New Dammarane-Type Acetylated Triterpenoids and Their Related Compounds of *Ficus pumila* Fruit

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From the methanolic extract of *Ficus pumila* LINN. fruit (Moraceae), two new acetylated dammarane-type triterpenoids (3, 4) and their two new related compounds (1, 2) were obtained, and their structures were determined by spectral methods as 3β-acetoxy-22,23,24,25,26,27-hexanordammaran-20-one (1), 3β-acetoxy-20,21,22,23,24,25,26,27-octanordammaran-17β-ol (2), 3β-acetoxy-(20*R*,22*E*,24*RS*)-20,24-dimethoxydammaran-22-en-25-ol (3) and 3β-acetoxy-(20*S*,22*E*,24*RS*)-20,24-dimethoxydammaran-22-en-25-ol (4).

Key words *Ficus pumila* fruit; dammarane-type triterpenoid; hexanordammarane; octanordammarane; Moraceae

In continuation of our studies on the constituents of *Ficus* genus,[1)] we describe acetylated dammarane-type triterpenoids of the fruit of *Ficus pumila* LINN. (Moraceae; ohitabi in Japanese) which has been used in Chinese folk medicine as an anti-tumor, anti-inflammatory and tonic medicament.

The methanolic extract of the cut fresh fruit was worked up as described in the Experimental, and from the ether extract, new triterpenoids (**3**, **4**) and their related compounds (**1**, **2**) were obtained by repeated silica gel and Lobar RP-8 column chromatography, and HPLC using octadecyl silanized silica (ODS) and Symmetry Prep C_{18} columns.

Compound **1** ($C_{26}H_{42}O_3$, mp 76—79 °C, $[\alpha]_D^{23}$ +47.4°) showed the $[M]^+$ and $[M-C_2H_3O-CH_3COOH]^+$ ion peaks at *m*/*z* 402 and 299 in the EI-MS. The ^{1}H-, ^{13}C- and ^{13}C–^{1}H shift correlated spectroscopy (COSY) NMR spectral data (Tables 1, 2) of **1** revealed the presence of five *tert*-methyls, eight methylenes, five methines (one of them was oxygenated), four quaternary carbons, one methylketone group and one equatorial acetoxyl group. From the analysis of heteronuclear multiple-bond correlation (HMBC) spectral data, the partial structure in **1** (Fig. 1; shown in heavy lines) was obtained from the two or three bond correlations from the signals of six methyl protons and the H-17 proton. Then, **1** was considered to be a hexanor-derivative of the dammarane-type triterpenoid having an acetoxyl group at C-3 and carbonyl oxygen at C-20. Comparison of the ^{13}C-NMR data with those of dammarane-type triterpenoids[2)] allowed the assignment of the ^{13}C-NMR signal of **1** as shown in Table 2. Further, observed nuclear Overhauser effect (NOE) interactions between the proton signal of H_3-18 and H-13, and between H_3-30 and H-17 in its nuclear Overhauser enhancement spectroscopy (NOESY) spectrum (Fig. 2), indicate the configuration at H-13 and H-17 should be β and α, respectively. So, **1** could be characterized as 3β-acetoxy-22,23,24,25,26,27-hexanordammaran-20-one. Compound **1** is the nor-derivative of the triterpenoid corresponding to pregnane to the steroid.

Compound **2** ($C_{24}H_{40}O_3$, amorphous powder, $[\alpha]_D^{23}$ +14.4°) showed the $[M]^+$ ion peak at *m*/*z* 376 in the EI-MS. By comparison of NMR data with those of **1**, ^{1}H- and ^{13}C-NMR chemical shift could be assigned as Tables 1 and 2, and **2** was indicated to be an octanor-derivative of the dammarane-type triterpenoid having equatorial acetoxyl and hydroxyl groups at C-3 and C-17, respectively. In addition, NOE interaction between the H_3-30 and H-17 signals was observed in its NOESY spectrum (Fig. 2), thus, the configuration at H-17 should be α. Therefore, **2** could be characterized as 3β-acetoxy-20,21,22,23,24,25,26,27-octanordammaran-17β-ol.

Triterpenoid **3** ($C_{34}H_{58}O_5$, amorphous powder) and **4** ($C_{34}H_{58}O_5$, amorphous powder) showed the $[M+H]^+$ ion peaks at *m*/*z* 547 in the positive FAB-MS, and their formulae

Table 1. ^{1}H-NMR Spectral Data for **1** to **4**

	1	**2**
H-3	4.48 (dd, *J*=10.0, 6.0 Hz)	4.48 (dd, *J*=10.0, 6.0 Hz)
H-13	1.92 (m)	
H-17	2.59 (ddd, *J*=11.0, 11.0, 6.5 Hz)	3.92 (ddd, *J*=9.0, 9.0, 5.0 Hz)
H_3-18	0.98 (s)	0.99 (s)
H_3-19	0.87 (s)	0.85 (s)
H_3-21	2.13 (s)	
H_3-28	0.853 (s)	0.85 (s)
H_3-29	0.849 (s)	0.83 (s)
H_3-30	0.87 (s)	0.88 (s)
OAc	2.05 (s)	2.05 (s)

	3	**4**
H-3	4.48 (dd, *J*=10.0, 6.0 Hz)	4.48 (dd, *J*=10.0, 6.0 Hz)
H_3-18	0.93 (s)	0.90 (s)
H_3-19	0.86 (s)	0.86 (s)
H_3-21	1.23 (s)	1.219 (s)
		[1.221 (s)]
H-22	5.60 (d, *J*=16.0 Hz)	5.73 (d, *J*=16.0 Hz)
	[5.61 (d, *J*=16.0 Hz)]	[5.72 (d, *J*=16.0 Hz)]
H-23	5.39 (dd, *J*=16.0, 8.5 Hz)	5.41 (dd, *J*=16.0, 8.5 Hz)
	[5.40 (dd, *J*=16.0, 8.5 Hz)]	[5.42 (dd, *J*=16.0, 8.5 Hz)]
H-24	3.33 (d, *J*=8.5 Hz)	3.379 (d, *J*=8.5 Hz)
	[3.35 (d, *J*=8.5 Hz)]	[3.381 (d, *J*=8.5 Hz)]
H_3-26	1.135 (s)	1.15 (s)
	[1.143 (s)]	[1.16 (s)]
H_3-27	1.164 (s)	1.18 (s)
	[1.169 (s)]	[1.19 (s)]
H_3-28	0.85 (s)	0.84 (s)
H_3-29	0.85 (s)	0.84 (s)
H_3-30	0.86 (s)	0.86 (s)
OAc	2.04 (s)	2.04 (s)
20-OCH_3	3.13 (s)	3.15(s)
	[3.11 (s)]	[3.13 (s)]
24-OCH_3	3.304 (s)	3.33 (s)
	[3.299 (s)]	[3.31 s)]

Solvent: $CDCl_3$ (500 MHz). δ in ppm from TMS [coupling constants (*J*) in Hz are given in parentheses]. Mimor epimeric components are given in brackets.

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Table 2. ^{13}C-NMR Spectral Data for **1**—**4**

	1	**2**	**3**	**4**		**1**	**2**	**3**	**4**
C-1	38.78	38.80	38.72	38.70	C-20	221.44		80.16	80.49
C-2	23.69	23.70	23.70	23.70				[80.10]	[80.57]
C-3	80.87	80.88	80.93	80.94	C-21	30.00		18.31	20.59
C-4	37.90	37.90	37.89	37.89				[18.03]	[20.64]
C-5	55.95	55.97	55.92	55.91	C-22			140.87	139.22
C-6	18.13	18.09	18.14	18.13				[140.76]	[139.36]
C-7	35.46	34.48	35.17	35.14	C-23			127.08	128.00
C-8	40.50	40.22	40.43	40.40				[127.14]	[128.23]
C-9	50.61	50.84	50.61	50.58	C-24			89.69	89.76
C-10	37.11	37.14	37.05	37.04					[89.83]
C-11	21.24	21.10	21.46	21.43	C-25			72.17	72.15
			[21.49]					[72.13]	
C-12	25.97	24.10	25.43	25.04	C-26			24.31	24.29
C-13	45.12	50.17	42.67	43.03				[24.28]	
				[43.07]	C-27			26.19	26.22
C-14	50.05	48.37	50.05	49.08	C-28	27.97	27.98	27.97	27.95
C-15	31.55	30.06	30.99	30.77	C-29	16.50	16.51	16.49	16.49
				[30.85]	C-30	15.86	16.43	16.25	16.21
C-16	25.57	32.22	27.07	26.92	OAc	21.33	21.34	21.35	21.33
			[27.09]			171.05	171.05	171.01	171.01
C-17	54.26	76.44	49.25	49.73	20-OCH_3			50.00	49.93
			[49.14]	[49.83]	24-OCH_3			56.72	56.81
C-18	15.57	15.61	15.49	15.46				[56.82]	
C-19	16.30	16.33	16.28	16.26					

Solvent: $CDCl_3$ (125.65 MHz). δ in ppm from TMS. Mimor epimeric components are given in brackets.

were suggested from the accurate mass number of the $[M+H]^+$ ion peaks in high-resolution (HR) positive FAB-MS. The ^{1}H-, ^{13}C- and ^{13}C–^{1}H COSY NMR spectrum data of **3** and **4** (Tables 1, 2) revealed the presence of eight *tert*-methyls, eight methylenes, six methines (two of them were oxygenated), six quaternary carbons (two of them were oxygenated), one disubstituted double bond, one acetoxyl, one hydroxyl and two methoxyl groups, respectively.

Comparison of NMR data with those of **1**, **2** and some dammarane-type triterpenoids,[2,3] and the analysis of HMBC spectral data (Fig. 1; shown in heavy lines) suggested that **3** and **4** were dammarane-type triterpenoids having a double bond at C-22 (23), equatorial acetoxyl at C-3, tertiary hydroxyl at C-25 and methoxyl at C-20 and C-24. The ^{13}C signals of C-11, C-13, C-15 to C-17, C-20 to C-26 and the methoxyl attached to C-24 were recognized as a doubling peak in the ^{13}C-NMR spectra of **3** and **4**. The doubling signals suggested they existed as a mixture of C-24 epimers, and by the signal intensity of 20-OCH_3 and 24-OCH_3, the ratio of these epimers was clarified to be 3 : 2 for **3** and 4 : 3 for **4**, respectively. Comparison of NMR data also confirmed them to be 20-epimers.[4] As Asakawa *et al*. reported,[3] the ^{13}C chemical shifts of C-21 and C-22 of the 20-hydroxylated dammarane-type triterpenoid reflect the configuration at C-20, and in the case of dammarendiol, chemical shift differences between the pair of 20-epimers [20(*R*)–20(*S*)] showed $-\delta$ 1.4 (C-21) and $+\delta$ 1.3 (C-22), respectively. The ^{13}C-NMR spectra of **3** and **4** showed good similarity except the C-21 and C-22 signals, and the chemical shift differences of the C-21 and C-22 between **3** and **4** ($-\delta$ 2.28 [$-\delta$ 2.61] for C-21; $+\delta$ 1.65 [$+\delta$ 1.40] for C-22) showed good coincidence with that of 20(*R*)- and 20(*S*)-dammarendiol. Therefore, the configuration at C-20 was found to be *R* for **3** and *S* for **4**. Furthermore, the configuration of the double bond was found to be *E* by the broad coupling constants (16 Hz) between the olefinic protons in their ^{1}H-NMR spectra.[5] From these facts, **3** and **4** were characterized as 3β-acetoxy-(20*R*,22*E*,24*RS*)-20,24-dimethoxydammaran-22-en-25-ol (a mixture about 3 : 2) and 3β-acetoxy-(20*S*,22*E*,24*RS*)-20,24-dimethoxydammaran-22-en-25-ol (a mixture about 4 : 3), re-

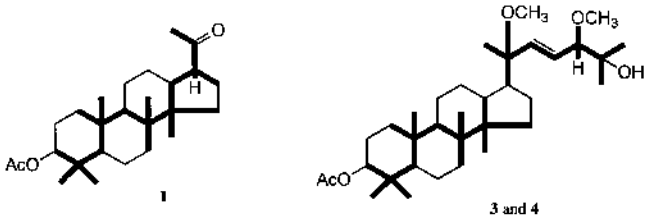

Fig. 1. Partial Structures of **1**, **3** and **4** Solved by HMBC Spectra

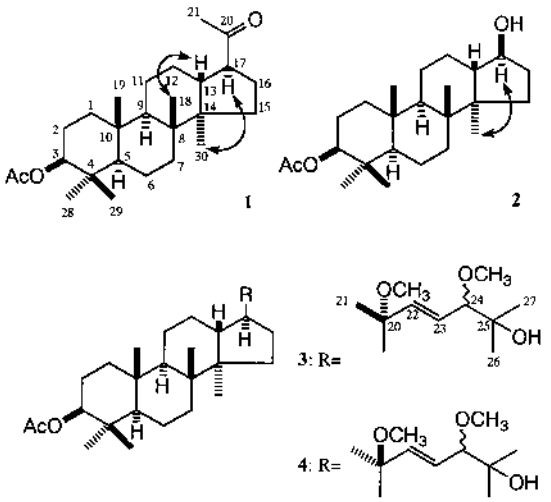

Fig. 2. Structures of **1**—**4**, and NOE Interactions Observed in the NOESY Spectra of **1** and **2**

spectively.

Experimental

The instruments and experimental conditions for obtaining spectral data for chromatography were the same as in the preceding papers.[1a)]

Extraction and Separation of 1 to 4 *F. pumila* LINN. was collected at Gushikawa City in Okinawa Prefecture, Japan, in March 1994. The fresh fruit (28 kg) was extracted with MeOH (32 l) at room temperature. After evaporation of the solvent, the residue (986.5 g) was suspended in water and successively extracted with ether, and the thus-obtained ether-soluble portion (43.1 g) was extracted with hot *n*-hexane (500 ml×2). The hot *n*-hexane soluble portion (34.4 g) was chromatographed on silica gel [*n*-hexane–EtOAc (9 : 1→4 : 1→7 : 3)→EtOAc→MeOH] which furnished eight fractions (frs. 1 to 8). Fraction 4 (3.3 g) was subjected to silica gel [*n*-hexane–EtOAc (4 : 1), benzene] column chromatography to give four fractions (frs. 4-1 to 4-4). Fraction 4-4 (480 mg) was purified by Sephadex LH-20 (MeOH), repeated silica gel [*n*-hexane–EtOAc (4 : 1), $CHCl_3$] and Lobar RP-8 [MeOH–H_2O (9 : 1)→MeOH] column chromatography to give **1** (52 mg). Fraction 6 (1.8 g) was treated with MeOH, and the MeOH soluble portion was purified by repeated silica gel [*n*-hexane–EtOAc (4 : 1), $CHCl_3$], Lobar RP-8 [MeOH–H_2O (9 : 1)→MeOH] column chromatography and HPLC [Symmetry Prep C_{18}; MeOH–H_2O (19 : 1)] to give **4** (11 mg). Fraction 7 (1.5 g) was treated with MeOH, and the MeOH soluble portion was subjected to silica gel [*n*-hexane–EtOAc (4 : 1→3 : 2), $CHCl_3$] to give four fractions (frs. 7-2-1 to 7-2-4). From fr. 7-2-2, **3** (10 mg) was isolated by repeated HPLC [Symmetry Prep C_{18}; MeOH and ODS; MeOH–H_2O (19 : 1)]. From fr. 7-2-3, **2** (6 mg) was isolated by silica gel [*n*-hexane–EtOAc (4 : 1→7 : 3)], Sephadex LH-20 (MeOH) column chromatography and HPLC (ODS; MeOH).

3β-Acetoxy-22,23,24,25,26,27-hexanordammaran-20-one (**1**): Colorless neddles, mp 76—79° (*n*-hexane–EtOAc), $[\alpha]_D^{23}$ +47.4° (c=0.5, $CHCl_3$). EI-MS *m*/*z*: 402.3111 $[M]^+$ (Calcd for $C_{26}H_{42}O_3$: 402.3131), 359 $[M-CH_3CO]^+$, 342 $[M-CH_3COOH]^+$, 299 $[M-C_2H_3O-CH_3COOH]^+$ (base), 189 $[M-C_{12}H_{21}O_3]^+$.

3β-Acetoxy-20,21,22,23,24,25,26,27-octanordammaran-20-one (**2**): Amorphous powder, $[\alpha]_D^{23}$ +14.4° (c=0.5, $CHCl_3$). EI-MS *m*/*z*: 376.2983 $[M]^+$ (Calcd for $C_{24}H_{40}O_3$: 376.2978), 358.2885 $[M-H_2O]^+$ (Calcd for $C_{24}H_{38}O_2$: 358.2872), 343 $[M-H_2O-CH_3]^+$, 316.2754 $[M-CH_3COOH]^+$ (Calcd for $C_{22}H_{36}O$: 316.2764), 298 $[M-CH_3COOH-H_2O]^+$, 189 $[M-C_{10}H_{19}O_3]^+$ (base).

3β-Acetoxy-(20*R*,22*E*,24*RS*)-20,24-dimethoxydammaran-22-en-25-ol (**3**): Amorphous powder. Positive FAB-MS *m*/*z*: 547.4328 $[M+H]^+$ (base, Calcd for $C_{34}H_{59}O_5$: 547.4363).

3β-Acetoxy-(20*S*,22*E*,24*RS*)-20,24-dimethoxydammaran-22-en-25-ol (**4**): Amorphous powder. Positive FAB-MS *m*/*z*: 547.4376 $[M+H]^+$ (base, Calcd for $C_{34}H_{59}O_5$: 547.4363).

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References and Notes

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