Triterpenoid Constituents of *Ficus thunbergii*

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Two new triterpenoids, rhoiptelenol and 3α-hydroxy-isohop-22(29)-en-24-oic acid, were isolated from the fresh leaves and stems of *Ficus thunbergii* MAX. (Moraceae), and their structures were determined by spectral and chemical methods. Along with these new compounds, known triterpenoids lupenyl acetate, β-amyrin acetate, α-amyrin acetate, lupeol, β-amyrin, α-amyrin, taraxerol, glutinol, ursolic acid and betulinic acid were obtained from the leaves and stems.

Keywords *Ficus thunbergii*; triterpenoid; rhoiptelenol; 3α-hydroxy-isohop-22(29)-en-24-oic acid; Moraceae; glutinol

Ficus thunbergii MAX. (Moraceae; himeitabi in Japanese) has been used in Chinese and Japanese folk medicine as an anti-rheumatalgia, anti-arthralgia and a drug for low back pain. To date, however, no report has been published about the ingredients of this plant. In this paper, we wish to describe the isolation and characterization of the triterpenoid components.

The methanol extract of fresh leaves of *F. thunbergii* was dissolved in water and extracted by ether. The ether extract was subjected to a combination of silica gel and alumina column chromatographies using a hexane–EtOAc solvent system and, finally, monoacetate, monool-I, monool-II, acid-I and acid-II parts were obtained as triterpenoid fractions. The monoacetate fraction was separated into three components by HPLC, and these were identified as lupenyl acetate (**1**), β-amyrin acetate (**2**), and α-amyrin acetate (**3**), respectively, by comparison of ^{1}H-NMR spectra with those of authentic samples.

The monool-I fraction was separated into four components by recrystallization and HPLC after acetylation. They were identified as taraxerol (**4**), lupeol (**5**), β-amyrin (**6**) and α-amyrin (**7**) by a method similar to that used for **1** to **3**.

Next, the monool-II fraction was separated into two components by HPLC after acetylation. The minor component was identified as glutinol (**8**)[1] by ^{1}H- and ^{13}C-NMR spectra of the alcohol and its acetate. The main component of this fraction was a new triterpenoid, rhoiptelenol (**9**) having a rearranged ursane skeleton.

Rhoiptelenol (**9**), mp 219—221 °C, $[\alpha]_D$ +63°, showed a M^+ ion peak at *m/z* 426 ($C_{30}H_{50}O$) and its ^{1}H-NMR spectrum showed the presence of a trisubstituted double bond, an axial OH group, six tertiary methyl groups and two secondary methyl groups (Table I). The ^{13}C-chemical shifts of A, B and C ring carbons and attached methyl carbons of **9** were close to those of **8**, indicating that those rings possess an identical substitution pattern to that in **8** (Table II).

The electron impact-MS (EI-MS) spectrum of **9** showed a fragmentation pattern very similar to that of **8**, and typical fragments, *m/z* 134 ($C_{10}H_{14}$), 152 ($C_{10}H_{16}O$) and 274 ($C_{20}H_{34}$), which were caused by an allylic cleavage, indicate that the trisubstituted double bond is located at C-5 in ring B.

The structure of the compound was finally established

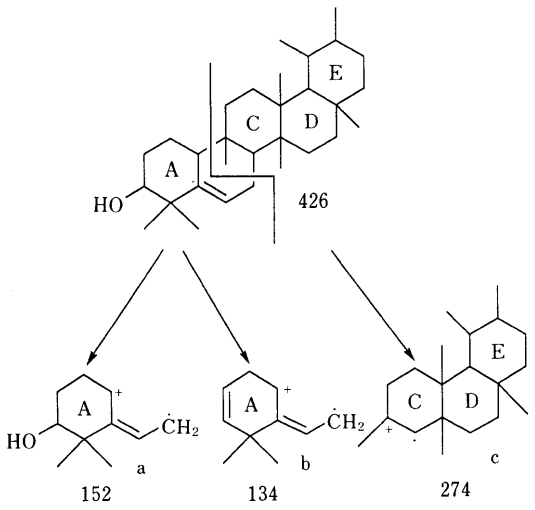

Chart 1. EI-MS Fragmentation of **9**

TABLE I. ^{1}H-Chemical Shifts (δ) of **9**, **10**, **12**—**15**

	Methyl signals of C-											
	23	24	25	26	27	28	29	30	3-H	Olefinic protons	Acetoxyl methyl	Methoxyl
9	1.047	1.142	0.896	0.993	0.921	1.065	0.987 d (6.7)	0.898 d (5.8)	3.47 br s	5.62 m [6]		
10	1.085	1.045	0.893	1.001	0.923	1.068	0.989 d (6.1)	0.889 d (6.1)	4.62 dd (2.4, 3.7)	5.54 m [6]	2.015	
12	1.788	—	1.180	1.112	0.941	0.697	—	1.706	4.73 br s	4.84 br s [29-H_2]		
13	1.219	—	0.782	1.014	0.991	0.694	—	1.678	5.28 br s	4.68, 4.70 br s [29-H_2]	2.100	
14	1.525	—	0.659	0.991	0.949	0.681	—	1.673	4.08 br s	4.68, 4.69 br s [29-H_2]		3.645
15	1.155	—	0.674	1.001	0.988	0.692	—	1.676	5.30 br s	4.68, 4.69 br s [29-H_2]	2.098	3.658

Solvent: $CDCl_3$ for **9**, **10**, **13**—**15**; C_5D_5N for **12**.

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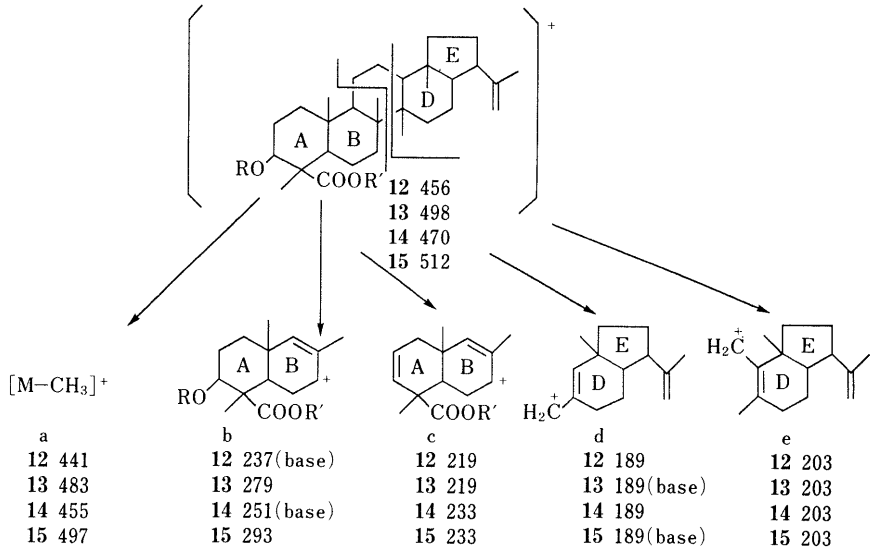

Chart 2. EI-MS Fragmentation of **12**, **13**, **14** and **15**

TABLE II. ^{13}C-Chemical Shift (δ)[a] of **8**—**10**, **12**—**15**

	8	9	10	12	13	14	15
C-1	23.66	23.92	23.77	34.89	34.42	33.85	34.47
C-2	18.23	18.11	18.82	24.30	23.95	26.29	23.96
C-3	76.35	76.36	78.60	70.69	73.34	71.11	73.63
C-4	39.32	38.76	39.10	48.42	46.70	47.57	46.86
C-5	141.63	141.80	142.11	49.22	50.37	48.77	50.37
C-6	122.07	122.13	120.08	20.70	19.61	19.82	19.67
C-7	27.84	27.77	25.43	33.86	33.13	33.13	33.12
C-8	43.09	45.30	45.22	42.07	41.70	41.67	41.68
C-9	34.86	34.74	34.68	50.09	49.62	49.56	49.56
C-10	49.76	49.76	49.90	38.28	37.66	37.51	37.42
C-11	34.61	34.25	34.27	21.57	21.25	21.23	21.24
C-12	30.37	28.34	28.33	24.30	23.71	23.94	23.78
C-13	37.85	38.76	38.76	49.01	48.70	48.66	48.70
C-14	39.83	39.91	39.91	42.53	42.36	42.32	42.36
C-15	32.10	28.36	28.36	32.92	32.66	32.56	32.65
C-16	36.03	34.86	34.83	20.70	20.88	20.84	20.88
C-17	30.11	31.37	31.39	54.21	53.88	53.83	53.89
C-18	47.45	52.38	52.41	44.40	44.20	44.17	44.21
C-19	35.09	35.72	35.73	40.36	40.21	40.17	40.17
C-20	28.26	31.92	31.93	27.61	27.37	27.34	27.37
C-21	33.13	29.76	29.74	48.20	47.92	47.86	47.91
C-22	38.97	37.69	37.69	148.09	148.19	148.22	148.19
C-23	28.98	29.00	29.15	25.37	23.71	23.94	23.60
C-24	25.45	25.46	25.04	180.67	181.62	177.67	176.46
C-25	16.20	17.18	17.01	14.21	13.26	13.16	13.22
C-26	18.43	15.39	15.32	16.81	16.57	16.54[b]	16.53
C-27	19.63	15.07	15.05	16.65	16.65	16.52[b]	16.63
C-28	32.41	38.76	38.76	15.33	15.18	15.17	15.18
C-29	34.53	25.22	25.24	110.06	109.50	109.46	109.48
C-30	31.93	22.48	22.49	19.74	19.68	19.66	19.67
OAc			170.89		170.38		170.39
			21.24		21.37		21.37
COOMe						51.18	51.43

a) Solvent: $CDCl_3$ for **8**—**10**, **13**—**15**; C_5D_5N for **12**. b) Assignment may be reversed.

as **9** by the results of heteronuclear multiple bond correlated (HMBC) and two dimensional (2D)-nuclear Overhauser enhancement spectroscopy (NOESY) NMR of the acetate (**10**). As the cross peaks between 18-βH and 26, 28 and 29 methyl groups were observed by NOESY, configuration of these methyl groups were considered to

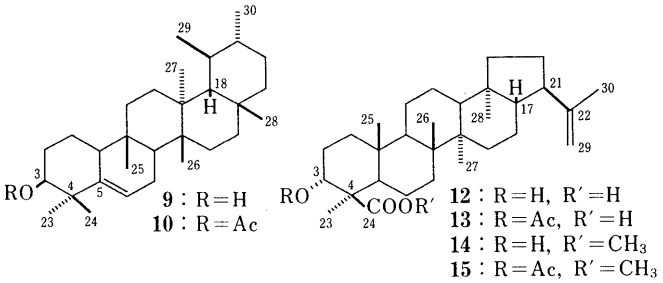

Chart 3

be β as true of other ursane triterpenoids.

This is the second example of a triterpenoid having Δ^5 rearranged ursane skeleton.[2]

Ursolic acid (**11**) was obtained from the acid-I fraction, and it was identified with authentic sample by ^{1}H- and ^{13}C-NMR spectra.

From the acid-II fraction, another new triterpenoid **12**, mp >300 °C, $[\alpha]_D$ +40°, M^+ at *m/z* 456 ($C_{30}H_{48}O_3$), was obtained as colorless needles. Its ^{1}H- and ^{13}C-NMR spectra showed the presence of an *exo*-methylene group, six tertiary methyl groups, an axial OH group and a carboxyl group, and suggested that **12** has a lupane or hopane skeleton with 3α-OH. The EI-MS spectra of **12**, acetate (**13**), methylate (**14**) and acetoxyl methylate (**15**) showed typical fragments, b and c, which were caused by bond cleavages of ring C, as shown in Chart 2. Thus, the carboxyl group was considered to be located at one of the *gem*-dimethyl groups of C-4.

The structure of **12** was determined by HMBC NMR spectroscopy of **13** and by the following evidence: 1) The ^{13}C-chemical shifts of D and E ring carbons and attached isopropenyl carbons of **13** were close to those of isohop-22(29)-ene.[3] 2) The ^{13}C-chemical shifts of A ring carbons and methyl and carboxyl carbons attached to C-4 of **13** were not identical to those of 3α-acetoxy-lup-20(29)-ene-23,28-dioic acid.[4] 3) Comparison of the ^{1}H-NMR spectrum of **15** with that of *epi*-betulinic acid acetate showed that the methyl signals assigned to 25-H_3 of **15**

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appeared further upfield (−0.18 ppm) than those of *epi*-betulinic acid acetate.[5] This would indicate a 1,3-diaxial disposition of $COOCH_3$ and CH_3, so that the position of carboxyl group must be C-24. From these results, **12** was established as 3α-hydroxy-isohop-22(29)-en-24-oic acid.

We also examined the constituents of the fresh stems of this plant and made a comparative study.

The methanol extract of fresh stems was treated in the same way as the fresh leaves, and **1**, **2**, **3**, **5**, **6**, **7**, **12** and betulinic acid (**16**) were detected as the triterpenoid constituents. They were identified by samples isolated from leaves or authentic samples, respectively.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-140 automatic polarimeter at 22 °C. MS were recorded with a JEOL JMS D-300 and a HX-110 spectrometer. ^{1}H- and ^{13}C-NMR spectra were taken on JEOL JNM GX-270 and GSX-500 spectrometers in $CDCl_3$ or C_5D_5N with tetramethylsilane as an internal standard, and chemical shifts are recorded in δ value. ^{1}H–^{13}C correlation spectroscopy (COSY) and HMBC were obtained with the usual pulse sequence and data processing was performed with standard JEOL software. Column chromatography was carried out under TLC monitoring using Kieselgel 60 (70—230 mesh, Merck), Silica Woelm TSC (silica gel for dry column, Woelm), aluminum oxide neutral (grade III, Woelm) and Sephadex LH-20 (25—100 μm, Pharmacia). TLC was performed on silica gel (Merck 5721) and spots were detected with anisaldehyde reagent. HPLC separation was carried out on a JASCO chromatograph (880-system) with a JASCO 830 RI detector and ODS-3251-D [Senshu pack; column size, 8 × 250 mm], and solvent used was CH_3CN : $CHCl_3$ = 4 : 1.

Extraction and Separation of Leaf Triterpenoids *F. thunbergii* MAX. was collected at Nago city in Okinawa prefecture of Japan in February, 1991. The fresh leaves (0.69 kg) were extracted with methanol (6.5 l) at room temperature. After evaporation of solvent, the residue (134.3 g) was dissolved in water and extracted with ether. Removal of the solvent gave the ether extract (16.9 g), and this was chromatographed on silica gel (*n*-hexane : EtOAc = 9 : 1 → 1 : 1, EtOAc, MeOH) which furnished eight fractions. Fraction 2 (monoacetate, 2.20 g) was purified by repeated silica gel (*n*-hexane : EtOAc = 19 : 1) and alumina (*n*-hexane : EtOAc = 24 : 1) column chromatography to afford a monoacetyl triterpenoid mixture (220 mg). The mixture (50 mg) was subjected to HPLC to give **1** (28 mg), **2** (10 mg) and **3** (12 mg). Fraction 3 (monool-II, 1.62 g) was purified by repeated silica gel (*n*-hexane : EtOAc = 4 : 1) and alumina (*n*-hexane : EtOAc = 9 : 1) column chromatography to afford a mixture of **8** and **9** (35 mg). The mixture was acetylated with Ac_2O and pyridine, and **8**-acetate (15 mg) and **10** (24 mg) were isolated by HPLC. **10** was hydrolyzed to **9** by treatment with 3% NaOH–90% tetrahydrofuran (THF) in the usual way. Fraction 4 (monool-I, 2.21 g) was chromatographed on silica gel (*n*-hexane : EtOAc = 4 : 1) to afford a monohydroxy triterpenoid mixture. The mixture was recrystallized from methanol and **4** (40 mg) was obtained as colorless needles. From the mother liquor, a mixture of **5**, **6** and **7** (230 mg) was obtained and the acetylated mixture (20 mg) was subjected to HPLC to give **1** (10 mg), **2** (4 mg) and **3** (6 mg). From fr. 6 (acid-I, 0.97 g), **11** (20 mg) was isolated by silica gel column chromatography ($CHCl_3$: MeOH = 19 : 1). Fraction 7 (0.10 g) was purified by silica gel ($CHCl_3$: MeOH = 19 : 1) and Sephadex LH-20 (MeOH) to afford **12** (5 mg).

Lupenyl Acetate (**1**): Colorless needles, mp 214—215 °C. The result of ^{1}H-NMR spectrum was identical with that of authentic sample.

β-Amyrin Acetate (**2**): Colorless needles, mp 242—244 °C. The result of ^{1}H-NMR spectrum was identical with that of authentic sample.

α-Amyrin Acetate (**3**): Colorless needles, mp 223—225 °C. The result of ^{1}H-NMR spectrum was identical with that of authentic sample.

Taraxerol (**4**): Colorless needles, mp 280—282 °C. The result of ^{1}H-NMR spectrum was identical with that of authentic sample.

Glutinol (**8**): Colorless needles, mp 208—210 °C. From the results of ^{1}H- and ^{13}C-NMR spectra of **8** and **8**-acetate (colorless needles, mp 189—190 °C), this was identified as glutinol.

Rhoiptelenol (**9**): Colorless needles, mp 219—221 °C, $[\alpha]_D$ +63° (c = 0.16, $CHCl_3$). EI-MS *m/z*: 426.3878 [$M(C_{30}H_{50}O)]^+$, 274.2468 ($C_{20}H_{34}$, c, base), 152.1270 ($C_{10}H_{16}O$, a), 134.0945 ($C_{10}H_{14}$, b). ^{1}H-NMR ($CDCl_3$) δ: Table I. ^{13}C-NMR ($CDCl_3$) δ: Table II.

9-Acetate (**10**): Colorless needles, mp 205—207 °C, $[\alpha]_D$ +73° (c = 0.22, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: Table I, 1.342 (br d, J = 2.2 Hz, 18β-H). ^{13}C-NMR ($CDCl_3$) δ: Table II.

Ursolic Acid (**11**): White powder (mp 288—290 °C). The results of ^{1}H- and ^{13}C-NMR spectra were identical with those of authentic sample.

3α-Hydroxy-isohop-22(29)-en-24-oic Acid (**12**): Colorless needles, mp > 300 °C, $[\alpha]_D$ +40° (c = 0.12, MeOH). EI-MS *m/z*: 456.3584 [$M(C_{30}H_{48}O_3)]^+$, 441.3464 ($C_{29}H_{45}O_3$, a), 237.1508 ($C_{14}H_{21}O_3$, b, base), 219.1472 ($C_{14}H_{19}O_2$, c), 203.1764 ($C_{15}H_{23}$, e), 189.1650 ($C_{14}H_{21}$, d). ^{1}H-NMR (C_5D_5N) δ: Table I. ^{13}C-NMR (C_5D_5N) δ: Table II.

Acetylation and Methylation of **12**: **12** was acetylated with Ac_2O and pyridine at room temperature for 12 h to give an acetate (**13**); colorless needles mp 249—253 °C, $[\alpha]_D$ +7° (c = 0.71, $CHCl_3$). EI-MS *m/z*: Chart 2. ^{1}H-NMR ($CDCl_3$) δ: Table I. ^{13}C-NMR ($CDCl_3$) δ: Table II.

12 was dissolved in ether and the solution was treated with ethereal diazomethane. The product was recrystallized from MeOH to obtain a methylate (**14**); colorless needles, mp 219—221 °C. EI-MS *m/z*: Chart 2. ^{1}H-NMR ($CDCl_3$) δ: Table I. ^{13}C-NMR ($CDCl_3$) δ: Table II.

Acetoxyl methylate (**15**) was obtained from **13** by treatment with ethereal diazomethane; colorless needles, mp 232—235 °C. EI-MS *m/z*: Chart 2. ^{1}H-NMR ($CDCl_3$) δ: Table I. ^{13}C-NMR ($CDCl_3$) δ: Table II.

Extraction and Separation of Stem Triterpenoids The fresh stems (3.5 kg) were extracted with methanol (12 l) at room temperature. After evaporation of the solvent, the residue (174.3 g) was dissolved in water and extracted with ether. Removal of the solvent gave the ether extract (13.5 g) and this was chromatographed on silica gel (*n*-hexane : EtOAc = 9 : 1 → 1 : 1, EtOAc, MeOH) which furnished eight fractions; these fractions were purified in the same way as described for leaves. Then, from fr. 2 (monoacetate, 1.04 g) and fr. 4 (monool-I, 0.80 g) a mixture of **1**, **2**, **3** (in the ratio of 20 : 14 : 1, 250 mg) and a mixture of **5**, **6**, **7** (in the ratio of 20 : 7 : 9, 110 mg) were obtained, respectively. From the acid fractions [fr. 6 (acid-I, 0.76 g) and fr. 7 (acid-II, 0.61 g)], **16** (110 mg) and **12** (90 mg) were obtained.

Betulinic Acid (**16**): Colorless needles, mp 270—273 °C. The results of ^{1}H- and ^{13}C-NMR spectra were identical with those of authentic sample.

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