Constituents of *Ficus pumila* Leaves

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From a methanolic extract of fresh leaves of *Ficus pumila* LINN (Moraceae), a new tocopherol-related compound has been isolated together with α-tocopherol, and its structure determined by spectral methods. Along with these compounds, two known sterols, fifteen known triterpenoids and five known flavonoid glycosides were identified as constituents.

Key words *Ficus pumila* leaf; Moraceae; α-tocopherol-related compound; triterpenoid; flavonoid glycoside

In a previous paper,[1] we reported the isolation and characterization of two new triterpenoids, rhoiptelenol and 3α-hydroxyisohop-22(29)-en-24-oic acid, together with ten known triterpenoids from the fresh leaves and stems of *Ficus thunbergii* MAX. (Moraceae, "himeitabi" in Japanese). These are used in Chinese and Japanese folk medicine as anti-rheumatalgia and anti-arthralgia medication, and to treat low back pain. The leaf of the same genus tree, *F. pumila* LINN. ("ohitabi" in Japanese) is also used for the same purposes as *F. thunbergii* in China and Japan, and as far as the constituents of this leaf are concerned, rutin, mesoinositol, taraxeryl acetate, amyrin acetate, sitosterol, herniarin and bergapten have been reported.[2] Here, we report the isolation and characterization of one new tocopherol-related compound along with the identification of α-tocopherol, two sterols, fifteen triterpenoids and five flavonoid glycosides from the fresh leaves of *F. pumila*.

A methanolic extract of the fresh leaves was dissolved in water and successively extracted with ether, ethyl acetate and *n*-butanol. The ether extract was subjected to silica gel column chromatography to obtain nine fractions. From fr. 5 and fr. 9, β-sitosterol (**1**) and β-sitosterol β-D-glucopyranoside (**2**) were isolated. From fr. 2, to fr. 4, lupenyl acetate (**3**), β-amyrin acetate (**4**), α-amyrin acetate (**5**), lupeol (**6**), β-amyrin (**7**), α-amyrin (**8**), glutinol (**9**), rhoiptelenol (**10**), α-tocopherol (**18**), and a new tocopherol-related compound VE-FPL (**19**) were isolated, from fr. 6 and fr. 7, ursolic acid (**11**), betulinic acid (**12**), 3α-hydroxyisohop-22(29)-en-24-oic acid (**13**), 2α,3β-dihydroxyurs-12-en-28-oic acid (**14**), 3β,28-dihydroxylup-20(29)-ene (**15**), 3β,28-dihydroxyolean-12-ene (**16**) and 3β,28-dihydroxyurs-12-ene (**17**) were obtained by repeated silica gel column chromatography and HPLC using an octadecyl silica (ODS) column. From fr. 8, bergapten was isolated. **1** to **18** were identified by comparison of ^{1}H- and ^{13}C-NMR data with those of authentic samples.

A new tocopherol-related compound, VE-FPL (**19**; $C_{29}H_{50}O_4$, a colorless oil, $[\alpha]_D^{22}$ +156°) showed an $[M+H]^+$ ion peak at *m/z* 463 in the positive FAB-MS. The ^{1}H-, ^{13}C- and ^{13}C–^{1}H correlation spectroscopy (COSY) NMR spectral data for **19** showed the presence of four *tert*-methyls, four *sec*-methyls, eleven methylenes, three methines, three oxygenated quaternary carbons, one tetrasubstituted double bond and two carbonyl carbons. By comparison of the ^{1}H- and ^{13}C-NMR data with that of **18**, **19** was found to have a similar side-chain (C-3″ to C-16″). From the molecular formula and degree of unsaturation, **19** was considered to be a tocopherol-related compound with one carbonyl, one methylketone and one *tert*-hydroxyl group. The partial structures of **19** were obtained from the correlations of the heteronuclear multiple-bond correlation (HMBC) experiment which are shown in heavy lines in Fig. 1. Further, cross peaks between 7a-hydroxyl-H and C-1′, and C-2′ were observed in the HMBC spectrum, and nuclear Overhauser effect (NOE) interaction between the signals of 7a-hydroxyl-H and H-1″ was observed in its nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum (Fig. 2). This suggested that the 7a-hydroxyl-H and carbonyl group of C-1′ were connected by a hydrogen bond, and the configuration at the 7a-hydroxyl and 3a-methylketone group should have the same direction to 1″-methylene. Therefore, the structure of **19** was assigned as shown in Fig. 2 [3a-acetyl-2,3,5-trimethyl-7a-hydroxy-5-(4,8,12-trimethyltridecanyl)-1,3a,5,6,7,7a-hexahydro-4-oxainden-1-one] except for the absolute configuration.

From the ethyl acetate extracts, astragalin (kaempferol 3-*O*-β-D-glucopyranoside, **20**) and isoquercitrin (quercetin 3-*O*-β-D-glucopyranoside, **21**) were isolated. From the *n*-butanol extract, **20**, **21**, apigenin 6-*C*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**22**),[3] kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**23**) and kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**24**)[4] were obtained by repeated chromatography on silica gel and Sephadex LH-20, and HPLC using an ODS column.[5] **20**, **21** and **23** were identified by comparison of ^{1}H- and ^{13}C-NMR with those of authentic samples, and **22** and **24** were identi-

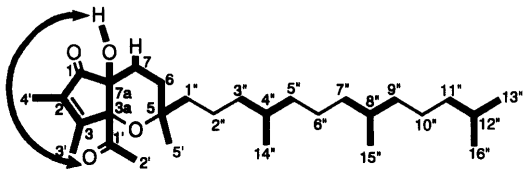

Fig. 1. Partial Structures of **19** Solved by HMBC Spectra (Heavy Lines)

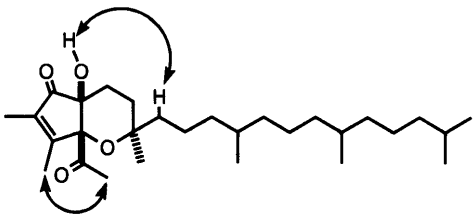

Fig. 2. Structure (Rel. Config.) and NOE Interactions Observed in the NOESY Spectra of **19**

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fied by comparison of ^{1}H- and ^{13}C-NMR data with the literature values, a ^{13}C-NMR experiment,[6] and analysis of acid hydrolyzed products.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-370 digital polarimeter. MS were recorded with a JEOL HX-110 spectrometer. ^{1}H- and ^{13}C-NMR spectra were run on JEOL JNM FX-100, GX-270 and A-500 spectrometers with tetramethylsilane as an internal standard, and chemical shifts are recorded in δ units. Column chromatography was carried out with TLC monitoring using Kieselgel 60 (70—230 mesh, Merck), Silica Woelm TSC (silica gel for dry column use, Woelm), aluminium oxide neutral (grade III, Woelm), Sephadex LH-20 (25—100 μm, Pharmacia), a Lobar RP-8 column (Merck) and Amberlite XAD-II (Organo). TLC was performed on silica gel (Merck 5721) and spots were detected with *p*-anisaldehyde–H_2SO_4 reagent. HPLC separation was carried out on a JASCO chromatograph (980-system) with a JASCO RI-930 detector and ODS-3251-D (Senshu pak; 8×250 mm), Symmetry Prep C18 (Waters; 7.8×300 mm) columns or a 930 Waters liquid chromatograph (204 type) with a Waters Lambda-Max Model 481 LC spectrophotometer and a Megapak SIL C_{18}-10 (JASCO; 7.5×250 μm) column.

Extraction and Separation of Leaf Constituents *F. pumila* Linn. was collected at Nago City in Okinawa Prefecture, Japan, in March 1994. The fresh leaves (2.78 kg) were extracted with methanol (16 l) at room temperature. After evaporation of the solvent, the residue (67.7 g) was successively partitioned between ether–water, ethyl acetate–water and *n*-butanol–water. Removal of the solvents from each phase gave ether (24.0 g), ethyl acetate (9.4 g), *n*-buthanol (23.4 g) and aqueous (10.9 g) extracts. The ether residue was chromatographed on silica gel [*n*-hexane–EtOAc (9 : 1→4 : 1→7 : 3→1 : 1)→EtOAc→MeOH] to give nine fractions. Fraction 2 (5.13 g) was purified by silica gel [*n*-hexane–EtOAc (19 : 1)] and alumina [*n*-hexane–EtOAc (24 : 1)] column chromatography to afford a monoacetyl triterpenoid mixture (910 mg) and **18** (25 mg). The mixture (30 mg) was subjected to HPLC [ODS; CH_3CN–$CHCl_3$ (4 : 1)] to give **3** (17 mg), **4** (7 mg) and **5** (2 mg). Fraction 3 (2.37 g) was subjected to repeated silica gel [*n*-hexane–EtOAc (4 : 1)] and alumina [*n*-hexane–EtOAc (9 : 1)] column chromatography to give a mixture of **9** and **10** (200 mg). The mixture (30 mg) was acetylated, and then separated into the **9**-acetate (10 mg) and **10**-acetate (17 mg) by HPLC [ODS; CH_3CN–$CHCl_3$ (4 : 1)]. Fraction 4 (2.45 g) was subjected to repeated silica gel [*n*-hexane–EtOAc (4 : 1)] and alumina [*n*-hexane–EtOAc (9 : 1)] column chromatography and HPLC (Symmetry Prep C_{18}; MeOH) to give **19** (9.5 mg) and a mixture of **6**, **7** and **8** (393 mg). The mixture (40 mg) was acetylated, and then separated into the **6**-acetate (10 mg), **7**-acetate (17 mg) and **8**-acetate (8 mg) by HPLC [ODS; CH_3CN–$CHCl_3$ (4 : 1)]. Fraction 5 (1.22 g) was subjected to silica gel chromatography [*n*-hexane–EtOAc (4 : 1)] and recrystallized from methanol to give **1** (234 mg). Fraction 6 (1.46 g) was treated with charcoal in methanol, and purified by repeated silica gel [*n*-hexane–EtOAc (7 : 3), $CHCl_3$–MeOH (19 : 1)] column chromatography to give **11** (78 mg) and **12** (125 mg). Fraction 7 (4.02 g) was also treated with charcoal in methanol, and subjected to silica gel [*n*-hexane–EtOAc (3 : 2), $CHCl_3$–MeOH (9 : 1)] and Sephadex LH-20 (MeOH) column chromatography to give **13** (33 mg), **14** (60 mg) and a mixture of **15**, **16** and **17** (50 mg). The mixture was separated by HPLC (ODS, CH_3CN) to give **17** (15 mg), and a mixture of **15** and **16** (27 mg). The latter was acetylated, and then separated to give the **15**-acetate and **16**-acetate by HPLC (ODS; CH_3CN). These were hydrolyzed to **15** (12 mg) and **16** (10 mg) by heating in a water bath with 3% NaOH–20% aq. MeOH for 2 h. Fraction 8 (1.42 g) was purified by silica gel [$CHCl_3$–MeOH (19 : 1)] and Sephadex LH-20 (MeOH) chromatography to give bergapten (125 mg). Fraction 9 (4.30 g) was purified by silica gel [$CHCl_3$–MeOH (9 : 1)] and Sephadex LH-20 (MeOH) chromatography to give **2** (56 mg).

The ethyl acetate residue was chromatographed on silica gel [$CHCl_3$–MeOH–H_2O (8 : 2 : 0.2→7 : 3 : 0.5)–MeOH] to give five fractions. Fraction 3 (0.43 g) was purified by silica gel [$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)] and Sephadex LH-20 (MeOH) chromatography to give **20** (118 mg). Fraction 4′ (0.34 g) was also purified by silica gel [$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)] and Sephadex LH-20 (MeOH) chromatography to give **21** (72 mg). The *n*-butanol extract was subjected to column chromatography on Amberlite XAD-II (H_2O→MeOH) and the methanol eluate (13.5 g) was chromatographed on Sephadex LH-20 (MeOH) to give five fractions. Fraction 2″ (0.94 g) was purified by repeated silica gel [$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)], Sephadex LH-20 (MeOH) and Lobar RP-8 [MeOH–H_2O (1 : 1)] column chromatography to give **22** (65 mg). Fraction 3″ (3.49 g) was subjected to repeated silica gel [$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)] and Sephadex LH-20 (MeOH) chromatography to give **20** (28 mg) and a mixture of **23** and **24**. The mixture was separated by HPLC {Megapak SIL; [HCOOH–H_2O (1 : 19)]–MeOH (7 : 3)} to give **23** (45 mg) and **24** (65 mg). Fraction 4″ (1.54 g) was subjected to repeated silica gel [$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)] and Sephadex LH-20 (MeOH) chromatography to give **21** (47 mg).

The following compounds were identified by comparison with authentic samples.

β-Sitosterol (**1**), β-sitosterol β-D-glucopyranoside (**2**), lupenyl acetate (**3**; **6**-acetate), β-amyrin acetate (**4**; **7**-acetate), α-amyrin acetate (**5**), α-amyrin (**8**), gultinol (**9**), rhoiptelenol (**10**), ursolic acid (**11**), betulic acid (**12**). 3α-hydroxyisohop-22(29)-en-24-oic acid (**13**), 2α,3β-dihydroxyurs-12-en-28-oic acid (**14**), 3β,28-dihydroxylup-20(29)-ene (**15**), 3β,28-dihydroxyolean-12-ene (**16**), 3β,28-dihydroxyolurs-12-ene (**17**), astragalin (**20**), isoquercitrin (**21**), kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**23**), kaempferol 3-*O*-α-L-rhamnosyl-(1→6)-β-D-galactopyranoside (**24**).

α-Tocopherol (18) A pale yellow oil, $[\alpha]_D^{23}$ −54.5° (*c*=0.8, $CHCl_3$). Electron impact (EI)-MS *m/z*: 430 [M ($C_{29}H_{50}O_2$)]$^+$ (base), 205, 165. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 0.84 (3H, d, *J*=6.5 Hz, H_3-15″), 0.85 (3H, d, *J*=6.5 Hz, H_3-14″), 0.87 (6H, d, *J*=6.5 Hz, H_3-13″ and H_3-16″), 1.23 (3H, s, H_3-4′), 2.11 (6H, s, H_3-1′ and H_3-3′), 2.16 (3H, s, H_3-2′). ^{13}C-NMR (25 MHz, $CDCl_3$) δ: 118.5 (C-1), 144.5 (C-2), 121.0 (C-3), 122.6 (C-4), 145.5 (C-4a), 74.5 (C-6), 31.5 (C-7), 20.8 (C-8), 117.4 (C-8a), 11.8 (C-1′), 12.2 (C-2′), 11.3 (C-3′), 23.8 (C-4′), 39.8 (C-1″), 21.0 (C-2″), 37.5 (C-3″), 32.7 (C-4″), 37.3, 37.4, 37.4 (C-5″, C-7″ C-9″), 24.8 (C-6″), 32.8 (C-8″), 24.5 (C-10″), 39.4 (C-11″), 28.0 (C-12″), 22.6 (C-13″), 19.7, 19.8 (C-14″, C-15″), 22.7 (C-16″).

VE-FPF [3a-Acetyl-2,3,5-trimethyl-7a-hydroxy-5-(4,8,12-trimethyltridecanyl)-1,3a,5,6,7,7a-hexahydro-4-oxainden-1-one] (19) A colorless oil, $[\alpha]_D^{22}$ +156° (*c*=0.8, $CHCl_3$). Positive FAB-MS *m/z*: 463.3784 [M+H]$^+$ (base, Calcd for $C_{29}H_{51}O_4$: 463.3787). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.84, 0.85 (each 3H, d, *J*=6.5 Hz, H_3-14″ and H_3-15″), 0.87 (6H, d, *J*=6.5 Hz, H_3-13″ and H_3-16″), 1.05 (3H, s, H_3-5′), 1.61 (2H, dd, *J*=9.0, 7.0 Hz, H_2-1″), 1.70 (1H, ddd, *J*=13.5, 6.5, 6.0 Hz, H-6a), 1.80 (1H, ddd, *J*=13.0, 6.5, 6.0 Hz, H-7a), 1.82 (3H, d, *J*=1.0 Hz, H_3-3′), 1.84 (3H, d, *J*=1.0 Hz, H_3-4′), 1.91 (1H, ddd, *J*=13.5, 6.5, 6.0 Hz, H-6b), 2.02 (3H, s, H_3-2′), 2.44 (1H, ddd, *J*=13.0, 6.5, 6.0 Hz, H-7b), 4.73 (7a-OH). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 204.97 (C-1), 139.32 (C-2), 163.05 (C-3), 89.09 (C-3a), 87.07 (C-5), 36.18 (C-6), 32.89 (C-7), 92.20 (C-7a), 207.12 (C-1′), 24.48 (C-2′), 11.80 (C-3′), 8.70 (C-4′), 24.87 (C-5′), 41.48 (C-1″), 22.48 (C-2″), 37.30 (C-3″), 32.73 (C-4″), 37.48, 37.50, 37.54 (C-5″, C-7″, C-9″), 24.50 (C-6″), 32.81 (C-8″), 24.82 (C-10″), 39.38 (C-11″), 27.99 (C-12″), 22.74 (C-13″), 19.68, 19.77 (C-14″, C-15″), 22.64 (C-16″).

Apigenin 6-*C*-α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranoside (22) Yellow needles, mp 218—223°C (dec.), $[\alpha]_D^{22}$ −13° (*c*=1.0, MeOH). FAB-MS *m/z*: 579 [M($C_{27}H_{30}O_{14}$)+H]$^+$, 433 [M+H−146]$^+$. ^{1}H-NMR (270 MHz; pyridine-d_5) δ: 6.40, 6.46 (H-8), 6.88, 6.96 (H-3), 7.22 (br s, H-3′, H-5′), 7.83 (br s, H-2′, H-6′). ^{13}C-NMR (67.5 MHz; pyridine-d_5) δ: 164.40 (C-2), 103.75 (br, C-3), 183.10 (br, C-4), 162.55, 162.83 (C-5), 109.91, 110.96 (C-6), 164.90 (br, C-7), 94.10, 95.53 (C-8), 104.88 (br, C-9), 157.87 (br. C-10), 122.08 (C-1′), 128.85 (C-2′, C-6′), 116.65 (C-3′, C-5′), 162.65 (C-4′), glucosyl [73.76 (br, C-1), 81.20, 81.88 (C-2), 76.80, 77.42 (C-3), 71.73, 72.32 (C-4), 82.86 (br, C-5), 62.31, 63.38 (C-6)], rhamnosyl [102.53 (br, C-1), 72.60 (C-2, C-3), 73.54 (C-4), 69.77 (C-5), 18.76 (C-6)]; (pyridine-d_5, t=100 °C) δ: 164.75 (C-2), 104.25 (C-3), 183.08 (C-4), 161.95 (C-5), 110.09 (C-6), 164.94 (C-7), 95.41 (C-8), 105.34 (C-9), 158.20 (C-10), 122.80 (C-1c), 128.84 (C-2′, C-6′), 116.86 (C-3′, C-5′), 162.58 (C-4′), glucosyl [74.01 (C-1), 81.19 (C-2), 77.38 (C-3), 72.17 (C-4), 82.15 (C-5), 62.69 (C-6)], rhamnosyl [102.12 (C-1), 72.65 (C-2), 72.51 (C-3), 74.01 (C-4), 69.58 (C-5), 18.44 (C-6)].

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References and Note

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6) The ^{13}C-NMR spectrum of **22** measured at room temperature showed broad split signals of the C-5, C-6, C-8 of apigenin, the C-2, C-3, C-4, C-6 of glucose and broad singlet signals of the C-3, C-4, C-7, C-9, C-10 of apigenin, the C-1 and C-5 of glucose and the C-1 of rhamnose. When the spectra were measured at 100°C, these signals became sharp.