Oleanane Acid from *Myrica cerifera*

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From the twigs of *Myrica cerifera* L. (Myricaceae), a new oleanane triterpenic acid named myrica acid was isolated along with myricalactone and several other known constituents. The structure of the acid was determined as 3β-hydroxy-1-oxoolean-11,13(18)-dien-28-oic acid on the basis of chemical and spectral evidence.

Key words oleanane acid; myrica acid; *Myrica cerifera*; myricalactone; Myricaceae

Myricaceae plants are distributed in the coasts and poor lands of Japan, China, South-East Asia, Europe, America and the southern part of Africa.[1)] Two species, *Myrica rubra* and *M. gale* var. *tomentosa* are domestic to Japan, and from the Japanese Myrica plants we have reported the isolation and structure determination of several diarylheptanoids and triterpenoids.[2)] In a previous paper, we reported on myricalactone, a diketonic lactone of an oleanane type triterpene isolated from *M. gale* var. *tomentosa*.[3)]

Myrica cerifera (bayberry, wax myrtle) is a tall tree distributed in North America.[4)] The chemical constituents of the plant have been investigated, and diarylheptanoids, myricanone and *dl*-myricanol, and triterpenoids, myricadiol, taraxerol and oleanolic acid have been isolated.[5,6)] Recently Fujimoto *et al*.[7)] reported isolation of myriceric acids A, B, C, D from the plant. They also reported that myriceric acid A, a caffeoyl ester at C-27 of 27-hydroxy-3-oxoolean-12-en-28-oic acid, is a strong antagonist of the endothelin receptor.

In the course of our continuing research on the chemical constituents of *Myrica*,[2)] we isolated a new oleanane type triterpene named myrica acid (**1**) from the twigs of *M. cerifera* along with myricalactone (**2**) and several known constituents (Experimental). Myrica acid (**1**), mp 274—277 °C, $[\alpha]_D$ −57°, positive Liebermann–Burchard (L.B.) color reaction (reddish violet), was obtained from an acidic fraction of the methanolic extract through repeated chromatography on silica gel, and gave a monomethyl ester (**1b**) on treatment with diazomethane. The molecular formula of **1** was determined to be $C_{30}H_{44}O_4$ by HR-MS.

In the IR spectrum (KBr), **1** showed absorptions of hydroxyls (3500—2600 cm^{-1}), a carboxyl (1710 cm^{-1}) and a carbonyl (1701 cm^{-1}). In the ^{13}C-NMR spectrum of **1**, thirty carbon signals were observed including two carbonyls at δ_C 181.4 (COOH) and 212.2 (ketonic) ppm and two pairs of double bond carbons at 124.5, 130.0, 131.1 and 136.7 ppm. Seven methyl groups of **1** were observed as singlets at δ_H 0.80, 0.82, 0.95, 1.01, 1.035, 1.043 and 1.30 ppm in the ^{1}H-NMR, and so, they are all attached to quaternary carbons.

The molecular formula of **1** shows nine equivalents of unsaturation and four out of the nine were accounted for by two carbonyls and two double bonds. One oxygen atom out of the four in **1** was found to form a secondary alcohol, not an ether, because only one sp^3 carbon bearing an oxygen function was observed as a doublet at δ_C 78.9 ppm in the ^{13}C-NMR spectrum. It follows that **1** is a pentacyclic triterpene acid.

Compound **1** showed UV absorption maxima at 242 nm (log ε 4.32), 249 nm (log ε 4.37), 258 nm (log ε 4.22). The maxima did not show shifts on addition of acid nor alkali; namely **1** has a neutral chromophore. The maxima of the chromophore resemble those due to the olean-11,13(18)-diene system of myricalactone (λ_{max} 254, 262 nm) and especially of papiriogenin D (λ_{max} 243, 251, 260 nm).[8)] In the ^{1}H-NMR of **1**, two protons on double bonds were observed at δ_H 5.63 (J=2, 11 Hz) and 6.41 (J=3, 11 Hz) ppm. These protons are coupled with each other with a J value of 11 Hz to form a *cis* double bond, and are also coupled with a methine (C-9, δ_H 2.61, δ_C 46.2 ppm) from the result of ^{1}H–^{1}H and ^{13}C–^{1}H COSY spectra of **1**. The HMBC spectrum of **1** showed correlations between 9-H/C-11, 12, 13; 11-H/C-9, 13, 18; 12-H/C-9, 13 (Chart 1). On the basis of the above evidence, **1** has a partial structure A (Chart 1).

Compound **1** has a secondary alcoholic function (*vide supra*). The carbon forming the function which was observed at δ_C 78.9 ppm has a methine proton observed as a doublet of doublets (J=5, 12 Hz) at δ_H 3.50 ppm from the ^{13}C–^{1}H COSY spectrum of **1**. Since the ^{1}H–^{1}H COSY spectrum showed that the methine proton was coupled with two protons, which were both bound to a single carbon observed as a triplet at δ_C 44.0 ppm, at δ_H 2.45 (1H, dd, J=5, 12 Hz) and δ_H 3.15 ppm (1H, t, J=12 Hz) to form an ABX coupling pattern, a partial structure, 3β-hydroxy-1-one or 1β-hydroxy-3-one structure in the A ring of the oleanane skeleton, was assumed for **1**. The 3β-hydroxy-1-one structure (B in Chart 1) is reasonable for **1**, because the twin methyl groups (23-H$_3$, 24-H$_3$) have a long-range coupling with the alcoholic methine carbon, while the methyl group (25-H$_3$) is coupled with the ketonic carbon in the HMBC spectrum of **1**. The methylene protons at C-2 were experimentally exchangeable with deuterium atoms to form a dideuteriated **1** (**1a**) on alkaline treatment of **1** in methanol-d_4.

The HMBC spectrum showed that the protons at C-25 (δ_H 1.30 ppm) have a long-range coupling with the methine carbon at C-9 (δ_C 46.2 ppm, δ_H 2.61 ppm, *vide supra*), and each of the two protons at C-9 (δ_H 2.61 ppm) and C-12 (ethylenic proton, δ_H 5.63 ppm), with a quaternary carbon (δ_C 52.1 ppm, C-10) and 2-H$_2$, 25-H$_3$ and 5-H, also with the quaternary carbon of C-10. On the basis of the above HMBC spectral analysis of **1**, the partial structures A and B (Chart 1) were combined together to a new extended partial structure C.

The molecule **1** has an isolated methylene (t at δ_C 40.5

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Dedicated to the memory of Dr. Kyosuke Tsuda.

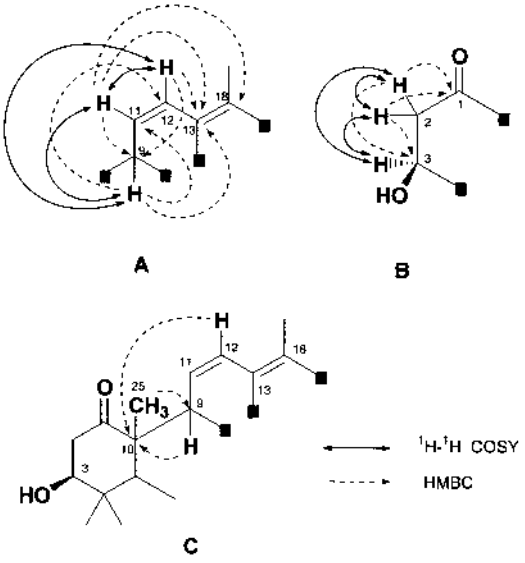

Chart 1

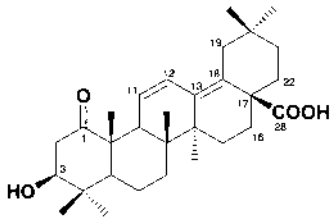

Chart 2

ppm; d at δ_H 1.66 ppm (J=14 Hz) and dd at δ_H 2.54 ppm (J=2, 14 Hz); position C-19). Since the methylene protons exhibited clear correlations with C-13 (double bond carbon), 17, 18 (double bond carbon), 20, 21 and 30 in the HMBC spectrum of **1**, the methylene is reasonably assigned to that at C-19 of an oleanane skeleton. Furthermore, in the spectrum, methylene (16-H_2, δ_H 1.73, 1.99 ppm) has long-range coupling with C-14, 22, 27 and 28 (COOH), and another methylene (22-H_2, δ_H 1.40, 2.27 ppm), with C-17, 18 (double bond carbon), 20, 28 (COOH), and 30. Each of the latter two methylenes was considered to form an isolated ethylene residue, judging from the ^{1}H–^{1}H COSY spectrum of **1**.

On the basis of all above findings, the structure of **1** was elucidated as 3β-hydroxy-1-oxoolean-11,13(18)-dien-28-oic acid (Chart 2).

Experimental

Instruments and TLC procedures used in this work were essentially the same as described in our previous papers.[2,3] NMR spectra (^{1}H with 500 MHz; ^{13}C with 125 MHz) were taken with a JMN GX 500 model, using tetramethylsilane (TMS) as internal standard. Chemical shifts were expressed in ppm and coupling constants (J-values) in Hz. s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet.

Plant Material Dried twigs of *Myrica cerifera* L. were purchased from Shinwa Bussan Co., Ltd., Osaka, Japan in 1995.

Extraction and Isolation The plant material (960 g) was extracted with MeOH (2.3 l), at room temperature three times. The combined MeOH solution (7 l) was concentrated under reduced pressure to afford the MeOH extract (57.4 g). The extract was suspended in EtOAc (200 ml) and filtered. The filtrate was concentrated under reduced pressure as far as possible to give an EtOAc soluble part (6.4 g). The EtOAc soluble part (1.0 g) was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, and concentrated. The residue (420 mg) tentatively called "$CHCl_3$ solubles" below, was dissolved in ether, and extracted with a saturated solution (50 ml) of $NaHCO_3$ in water, three times. The combined aq. solution was acidified with *dil*-HCl and extracted with ether. The ether solution, after concentrated to dryness, left a mixture (89 mg) of acidic compounds. The mixture of acidic compounds (total 0.5 g) was chromatographed on silica gel to give myricalactone (**2**, 13 mg) from fractions eluted with $CHCl_3$, myrica acid (**1**, 9.4 mg) from fractions eluted with $CHCl_3$–MeOH (100 : 3) and myriceric acid C (190 mg) from fractions eluted with $CHCl_3$–MeOH (100 : 7). Repeated chromatographic separation of "$CHCl_3$ solubles" above on silica gel provided myricanone, *dl*-myricanol, oleanolic acid, myricadiol, taraxerol and β-sitosterol. All the known compounds including myricalactone (**2**) were directly identified by comparison with authentic samples.

Compound 1 Colorless needles (MeOH), mp 274—277 °C, $[\alpha]_D$ −57° (c=0.8, MeOH), CD (c=2.50×10^{-5}, MeOH) $\Delta\varepsilon$ (nm): −29.05 (257), −46.89 (249), −40.73 (243). L.B. color reaction: positive (reddish violet). EI-MS m/z (%): 468 (33), 450 (41), 422 (63), 404 (100), 339 (94), 293 (41). HR-MS m/z: Calcd for $C_{30}H_{44}O_4$: 468.3239. Found: 468.3248. UV λ_{max} (MeOH) nm (log ε) : 242 (4.32), 249 (4.37), 258 (4.22, s). IR ν_{max} (KBr) cm^{-1}: 3500—2600, 1710, 1701, 1633, 1041, 968. ^{1}H-NMR ($CDCl_3$) δ: 0.80, 0.82, 0.95, 1.01, 1.035, 1.043, 1.30 (each 3H, s), 2.45 (1H, dd, J=5, 12 Hz, 2-Hα), 2.54 (1H, dd, J=2, 14Hz, 19-H), 2.61 (1H, br s, 9-H), 3.15 (1H, t, J=12 Hz, 2-Hβ), 3.50 (1H, dd, J=5, 12 Hz, 3-H), 5.63 (1H, dd, J=2, 11 Hz, 12-H), 6.41 (1H, dd, J=3, 11 Hz, 11-H). ^{13}C-NMR ($CDCl_3$) δ: 212.2 (CO-1), 44.0 (CH_2-2), 78.9 (CH-3), 39.4 (C-4), 53.2 (CH-5), 17.8 (CH_2-6), 31.7 (CH_2-7), 40.5 (C-8), 46.2 (CH-9), 52.1 (C-10), 124.5 (CH-11), 130.0 (CH-12), 136.7 (C-13), 42.3 (C-14), 25.0 (CH_2-15), 32.6 (CH_2-16), 48.0 (C-17), 131.1 (C-18), 40.5 (CH_2-19), 32.6 (C-20), 36.8 (CH_2-21), 35.5 (CH_2-22), 27.9 (CH_3-23), 15.3 (CH_3-24), 17.6 (CH_3-25), 16.7 (CH_3-26), 19.8 (CH_3-27), 181.4 (COOH-28), 32.2 (CH_3-29), 24.0 (CH_3-30).

Deuteriation of 1 A solution (0.75 ml) of 0.1% NaOMe-d_3 in MeOH-d_4 was added to a solution of **1** (2.0 mg) in MeOH-d_4 and kept overnight at room temperature. After removing the solvent under a N_2 stream the residue was acidified with 2.5% HCl and extracted with ether. The ether solution was washed with water, dried over anhydrous Na_2SO_4 and then concentrated to dryness. The residue was purified with chromatography on silica gel and elution with $CHCl_3$–MeOH (50 : 1) gave **1a** (0.7 mg). **1a**, colorless needles (MeOH), mp 273—275 °C. EI-MS m/z (%): 470 ($M^{+\cdot}$, 68), 452 (37), 405 (74), 340 (35), 293 (72), 248 (43), 199 (82).

Methylation of 1 A solution of **1** (2.0 mg) in MeOH (2 ml) was treated with diazomethane in ether in the usual manner. The product was purified by chromatography on silica gel ($CHCl_3$–MeOH (100 : 1)) to afford **1b** (1.2 mg). **1b**, colorless needles (MeOH), mp 196—198 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.79, 0.82, 0.92, 1.016, 1.024, 1.04, 1.31 (each 3H, s), 2.45 (1H, dd, J=5, 12 Hz), 2.52 (1H, dd, J=2, 14 Hz), 2.61 (1H, s), 3.15 (1H, t, J=12 Hz), 3.49 (1H, dd, J=5, 12 Hz), 3.66 (3H, s), 5.61 (1H, d, J=11 Hz), 6.41 (1H, dd, J=2, 11 Hz).

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