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Chemical Constituents of Chinese Natural Medicine, Morindae Radix, the Dried Roots of *Morinda officinalis* How.: Structures of Morindolide and Morofficinaloside

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A new iridoid lactone, morindolide, and a new iridoid glucoside, morofficinaloside, have been isolated from a Chinese natural medicine, Morindae Radix, the dried root of *Morinda officinalis* How. together with a number of known compounds: five anthraquinones, four iridoid glucosides, a monoterpene glycoside, two sterols, an ursane-type triterpene, and a lactone compound. The chemical structures of the new compounds were determined on the basis of chemical and physicochemical evidence.

Key words Morindae Radix; *Morinda officinalis*; Rubiaceae; morindolide; morofficinaloside; iridoid

The dried roots of *Morinda officinalis* How. (Rubiaceae) have been used as a Chinese natural medicine, Morindae Radix [Hagekiten (巴戟天) in Japanese], which is prescribed for tonic and analgesic purposes in Chinese traditional preparations. In chemical studies on the constituents of this natural medicine, two iridoid glucosides, monotropein (**10**) and asperuloside acetate, and several anthraquinones have been reported.[1] As a part of our chemical studies on biological active principles of natural medicines,[2] we have isolated two new constituents from Morindae Radix, namely an iridoid lactone and an iridoid glucoside designated as morindolide (**1**) and morofficinaloside (**2**), respectively, together with five anthraquinones, four iridoid glucosides, a monoterpene glycoside, two sterols, an ursane-type triterpene, and a lactone compound. This paper presents a full account of the isolation and structure elucidation of these constituents.[3]

The isolation of the chemical constituents from Morindae Radix was carried out through the procedure shown in Fig. 1. The methanolic extract of the roots was partitioned into an ethyl acetate and water mixture. The ethyl acetate-soluble portion was subjected to ordinary-phase and reversed-phase silica gel column chromatography, LH-20 column chromatography, preparative TLC, and high-performance liquid chromatography (HPLC) to furnish morindolide (**1**) together with tectoquinone (**3**),[4] alizarin 1-methyl ether (**4**),[5] lucidin-ω-methyl ether (**5**),[6] 1-hydroxy-2,3-dimethylanthraquinone (**6**),[7] and 1-hydroxy-3-hydroxymethylanthraquinone (**7**),[8] β-sitosterol, oxositosterol (**13**),[9] rotungenic acid (**14**)[10] and (4*R*,5*S*)-5-hydroxyhexan-4-olide (**15**).[11]

The water-soluble portion was extracted with 1-butanol and the 1-butanol-soluble portion was subjected to XAD-2 column chromatography to provide the methanol eluate and the water eluate. Repeated separation of the methanol eluate and water eluate by means of ordinary-phase and reversed-phase silica gel column chromatography and subsequent HPLC furnished morofficinaloside (**2**) together with asperuloside (**8**),[12] asperulosidic acid (**9**)[13] and *l*-borneol 6-*O*-β-D-apiosyl-β-D-glucoside (**12**)[14] from the methanol eluate and monotropein (**10**)[15] and desacetyl asperulosidic acid (**11**)[13] from the water eluate. This is the first time that the four anthraquinones (**4**—**7**), three iridoid glucosides (**8**, **9**, **11**), monoterpene glycoside (**12**), sterol (**13**), ursan-type triterpene (**14**), and lactone compound (**15**) have been isolated from Morindae Radix.

Morindolide (1) Morindolide (**1**) was obtained as a colorless oily substance. The molecular formula of **1** was determined to be $C_9H_{12}O_3$ from high-resolution MS measurement. In the positive mode FAB-MS of **1**, quasi-molecular ion peaks at *m/z* 191 $(M+Na)^+$ and *m/z* 169 $(M+H)^+$ were observed. The IR spectrum of **1** showed absorptions due to hydroxyl and lactone functions at 3450 and 1734 cm^{-1}. The ^1H-NMR and ^{13}C-NMR (Table 1) of **1** showed signals assignable to a trisubstituted olefin [δ 5.78 (br s, 7-H), δ_C 129.2, 140.0 (C-7, 8)], a lactone moiety [δ 4.27, 4.42 (both m, 3-H_2), δ_C 173.1, 67.6 (C-1, 3)], and a methylene bearing a hydroxyl group [δ 4.27 (br s, 10-H_2), δ_C 60.6 (C-10)], together with two methines [δ 2.88 (m, 5-H), 3.72 (d, J=9.5 Hz, 9-H)] and two methylenes. Acetylation of **1** with acetic anhydride in pyridine afforded the monoacetate (**1a**). The proton and carbon signals in the NMR spectra of **1a** were completely assigned with the aid of homo and hetero correlation spectroscopy (^1H–^1H, ^1H–^{13}C COSY), and distortionless enhancement by polarization transfer (DEPT). The connectivities of the quaternary carbons (C-1 and 8) were clarified by a heteronuclear multiple bond correlation (HMBC) experiment with **1a**. Namely, HMBC correlations were observed between the 1-carbon and the 3,5,9-protons and between the 8-carbon and the 7,9,10-protons as shown in Fig. 2, so that the iridoid lactone structure having 10-hydroxyl and 7,8-olefin groups was constructed. Furthermore, the stereostructure of morindolide (**1**) was characterized on the basis of the following nuclear Overhauser effect (2D-NOE) experiment on **1a** (Fig. 3), in which NOE correlation was observed between the signals of the 5-proton (δ 2.92) and 9-proton (δ 3.65). Consequently, the relative stereostructure of morindolide has been clarified as **1**.

Morofficinaloside (2) Morofficinaloside (**2**), obtained as a white powder, gave the quasi-molecular ion peak at *m/z* 405 $(M-H)^-$ in the negative mode FAB-MS and the molecular composition was defined as $C_{17}H_{26}O_{11}$ from

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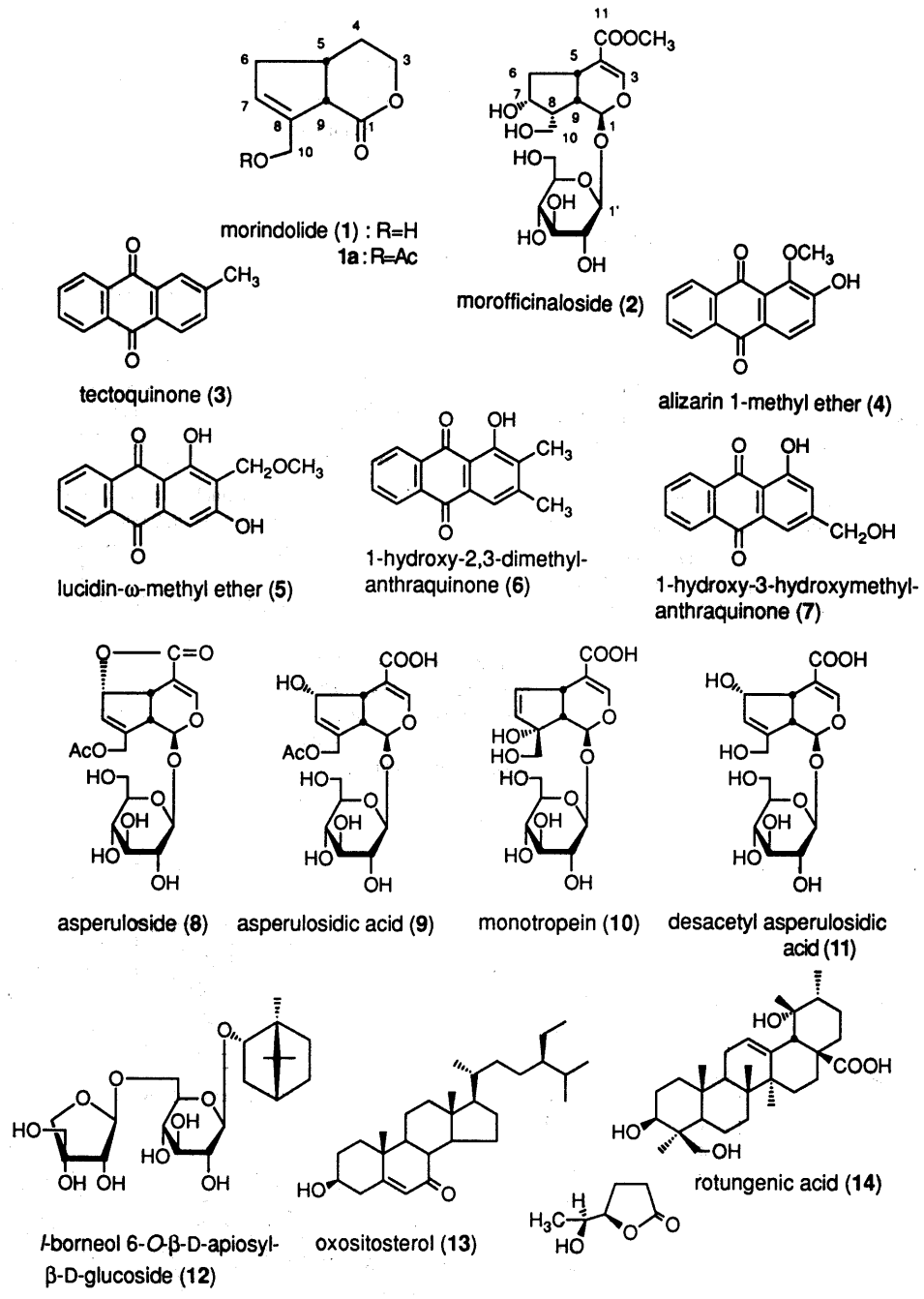

Chart 1

the high-resolution FAB-MS analysis. In the IR spectrum of **2**, it showed strong absorption bands at 3400 (br) and 1700 cm^{-1} indicative of glycoside structure and enone function. Morofficinaloside (**2**) showed a UV absorption maximum at 235 nm (log ε 5.08) which was ascribable to a conjugated enone chromophore. The ^{1}H-NMR spectrum of **2** showed the presence of a carbomethoxyl (δ 3.53, s), a methylene [δ 1.48 (ddd, J=5.0, 9.1, 13.9 Hz), 2.05 (m), 6-H_2], a hydroxy-bearing methylene [δ 3.55 (m), 3.70 (br d, J=10.6 Hz), 10-H_2], two methines [δ 1.95 (m, 9-H), 2.92 (m, 5-H)], a hydroxyl-bearing methine [δ 4.15 (m, 7-H)] and an acetal proton [δ 5.09 (d, J=4.6 Hz, 1-H)] together with a β-D-glucopyranosyl moiety [δ 4.63 (d, J=8.9 Hz, 1′-H)]. Detailed comparison of the ^{13}C-NMR data (Table 1) for **2** with those for known iridoid glucosides[16] led us to formulate the iridoid glucoside structure having 7,10-dihydroxyl groups for officinaloside (**2**). The stereostructure of **2** was characterized on the basis of the following nuclear Overhauser and exchange spectroscopy (NOESY) experiment: *i.e.*, cross peaks occurring between 1-H and 1′-H; 1-H and 10-H; 5-H and 9-H; 5-H and 7-H; 7-H and 8-H (Fig. 3). Based on those findings, the structure of morofficinaloside (**2**) was clarified.

Experimental

The instruments used for obtaining physical data and the experimental conditions for chromatography were the same as described in our previous paper.[2]

Extraction and Isolation Morindae Radix (4 kg, purchased from Honzo Seiyaku, Nagoya) was cut finely and extracted with MeOH under reflux three times. The MeOH extract (1.23 kg) obtained after removal

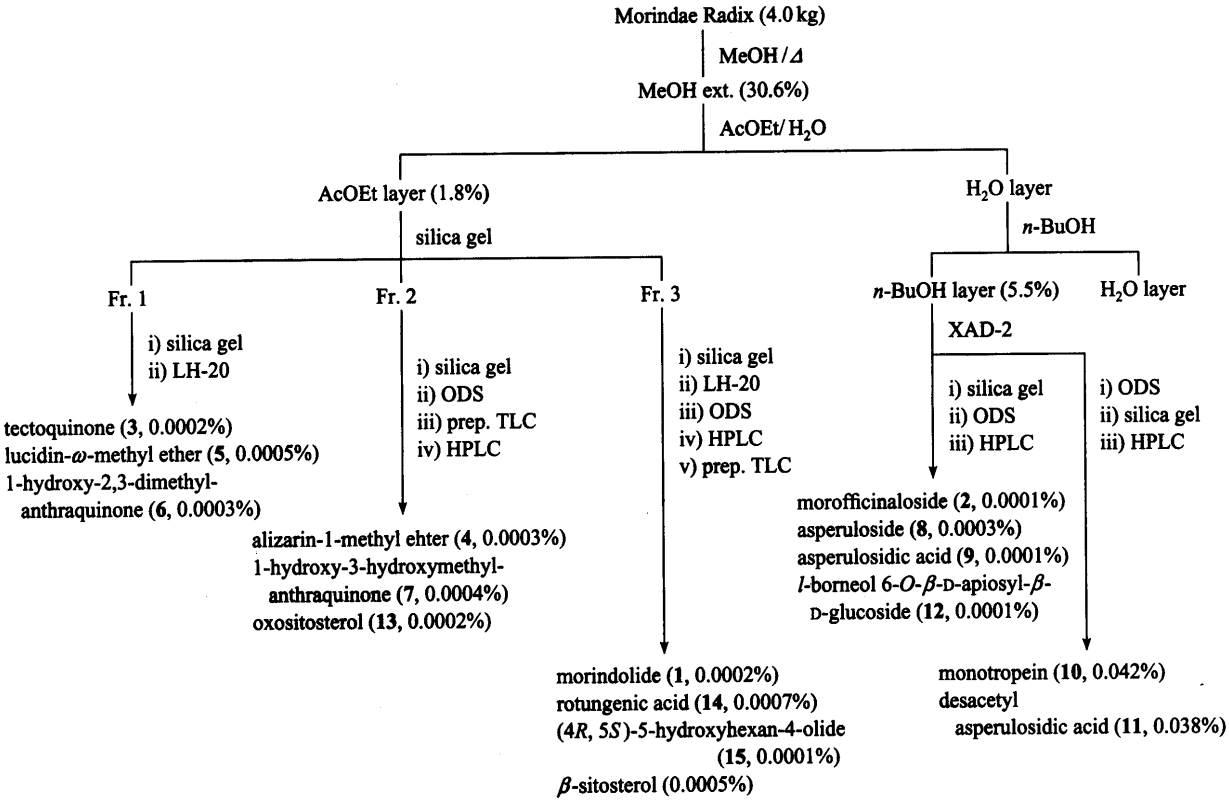

Fig. 1

Table 1. The ^{13}C-NMR Data for **1**,[a] **1a**,[a] and **2**[b]

	1	1a	2
C-1	173.1	171.1	98.5
C-3	67.6	66.8	152.6
C-4	29.2	29.7	112.9
C-5	34.7	34.6	31.8
C-6	38.8	39.1	41.5
C-7	129.2	131.4	72.6
C-8	140.0	135.8	48.3
C-9	50.5	50.4	41.8
C-10	60.6	61.9	61.3
C-11			170.9
OCH_3			52.6
C-1′			99.7
C-2′			73.5
C-3′			76.5
C-4′			70.3
C-5′			77.0
C-6′			61.4
OAc		20.1	
		170.6	

The spectra were taken *a*) in $CDCl_3$ at 75 MHz, or *b*) in D_2O at 68 MHz.

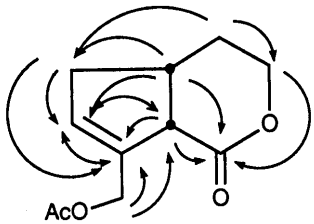

Fig. 2. HMBC Correlations of **1a**

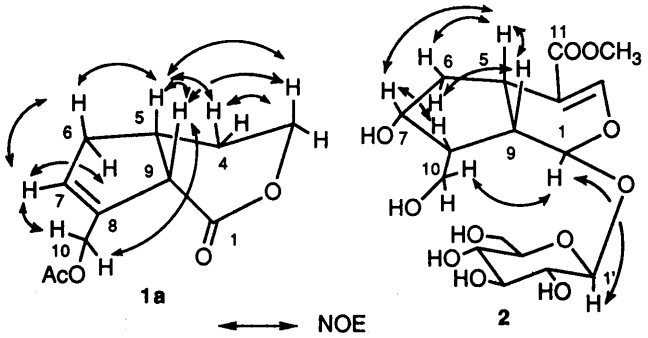

Fig. 3. NOE Correlations of **1a** and **2**

of the solvent *in vacuo* was partitioned into AcOEt–H_2O (1 : 1) solution. The aqueous layer was extracted with *n*-BuOH. Removal of the solvent *in vacuo* from the AcOEt-soluble portion and *n*-BuOH-soluble portion yielded 73.8 and 218 g of residue, respectively. The AcOEt extract (73.8 g) was subjected to SiO_2 column chromatography with *n*-hexane–AcOEt (4 : 1) to give three fractions (fractions 1—3). Fraction 1 (6.63 g) was purified successively by column chromatography [i) SiO_2, *n*-hexane–acetone; ii) Sephadex LH-20, $CHCl_3$–MeOH)] to afford **3** (31.4 mg), **5** (71.3 mg), and **6** (44.1 mg). Fraction 2 (4.78 g) was subjected to column chromatography [i) SiO_2, *n*-hexane–AcOEt, ii) SiO_2, benzene–AcOEt, iii) Chromatorex-ODS DM1020T, MeOH–H_2O], preparative TLC (pre-coated SiO_2 TLC plate, *n*-hexane–AcOEt), and then HPLC [Develosil 100-5 (JASCO), *n*-hexane–AcOEt] to give **4** (44.5 mg), **7** (54.2 mg) and **13** (25.2 mg).

Fraction 3 (7.51 g) was purified by column chromatography [i) SiO_2, $CHCl_3$–MeOH; ii) SiO_2, *n*-hexane–acetone; iii) Sephadex LH-20, $CHCl_3$–MeOH; iv) Chromatorex-ODS DM1020T 70% aqueous MeOH, v) Chromatorex-ODS DM1020T, 90% aqueous CH_3CN] and preparative TLC (precoated SiO_2 TLC plate, benzene–acetone) to furnish **1** (57.9 mg), **14** (87.0 mg), **15** (28.9 mg) and β-sitosterol (63.0 mg).

The *n*-BuOH extract (218 g) was separated by XAD-2 column chromatography (H_2O and MeOH) to give the MeOH-eluted fraction (17.4 g) and H_2O-eluted fraction (195.3 g). The MeOH eluate (17.4 g) was subjected to column chromatography [i) SiO_2, $CHCl_3$–MeOH–H_2O; ii) Chromatorex-ODS DM1020T, 50% aqueous MeOH] followed by HPLC (YMC Pack S-5 120A, 60% aqueous MeOH) to afford **2** (5.1 mg),

8 (10.3 mg), **9** (3.5 mg) and **12** (8.0 mg). The H_2O eluate (195.3 g) was purified by column chromatography [i) Chromatorex-ODS DM1020T, H_2O–MeOH; ii) SiO_2, $CHCl_3$–MeOH–H_2O] and HPLC (YMC Pack S-5 120A, 10% aqueous MeOH) to give **10** (1.68 g) and **11** (1.52 g). All known compounds (**3**—**15**) were identified by comparison of their physical data with reported values.[4-15]

Morindolide (**1**): A colorless oil, $[\alpha]_{22}^{D}$ −49.6° (c=0.6, EtOH). High-resolution FAB-MS: Found: 169.0850; Calcd for $C_9H_{13}O_3$ $(M+H)^+$: 169.0865. IR (KBr): 3450, 1734, 1400, 1076 cm^{-1}. CD (c=EtOH) $\Delta\varepsilon$: −14.9 (226). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.66 (1H, dddd, J=3.3, 8.4, 9.3, 16.0 Hz, 4β-H), 2.01 (1H, m, 4α-H), 2.12 (1H, m, 6α-H), 2.71 (1H, m, 6β-H), 2.88 (1H, m, 5-H), 3.72 (1H, br d, J=9.5 Hz, 9-H), 4.27 (1H, m), 4.42 (1H, m) (3-H_2), 4.27 (2H, br s, 10-H_2), 5.78 (1H, br s, 7-H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ_C: see Table 1. Positive FAB-MS m/z: 169 $(M+H)^+$, 191 $(M+Na)^+$.

Morofficinaloside (**2**): A white powder, $[\alpha]_D^{20}$ −40.0° (c=0.26, MeOH). High-resolution FAB-MS: Found: 405.1394; Calcd for $C_{17}H_{25}O_{11}$ $(M-H)^-$: 405.1497. UV λ_{max}^{MeOH} nm (log ε): 235 (5.08). IR (KBr): 3400, 1700, 1632, 1078 cm^{-1}. ^{1}H-NMR (D_2O, 270 MHz) δ: 1.48 (1H, ddd, J=5.0, 9.1, 13.9 Hz, 6β-H), 1.95 (1H, m, 9-H), 1.96 (1H, m, 8-H), 2.05 (1H, m, 6α-H), 2.92 (1H, m, 5-H), 3.06—3.65 (all 6H, sugar protons), 3.53 (3H, s, carbomethoxyl), 3.55 (1H, m), 3.70 (br d, J=10.6 Hz, 10-H_2), 4.15 (1H, m, 7-H), 4.63 (1H, d, J=8.9 Hz, 1'-H), 5.09 (1H, d, J=4.6 Hz, 1-H), 7.30 (1H, s, 3-H). ^{13}C-NMR (68 MHz): see Table 1. Negative FAB-MS m/z: 405 $(M-H)^-$.

Acetylation of 1 A solution of **1** (5.0 mg) in pyridine (0.5 ml) was treated with Ac_2O (0.5 ml) and the reaction mixture was stirred at room temperature under an N_2 atmosphere for 2 h. It was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with diluted aqueous HCl, aqueous $NaHCO_3$, and brine, and then dried over $MgSO_4$. After removal of the solvent from the AcOEt extract under reduced pressure, the product was purified by octadecyl silica (ODS) silica gel column chromatography (40% aqueous MeOH) to give **1a** (4.0 mg).

1a: A colorless oil. High-resolution FAB-MS: Found 211.0948. Calcd for $C_{11}H_{15}O_4$: 211.0970 $(M+H)^+$. IR (KBr): 3460, 1740, 1736, 1380, 1078 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.66 (1H, dddd, J=3.0, 7.0, 8.0, 15.0 Hz, 4β-H), 2.01 (1H, m, 4α-H), 2.07 (3H, s, OAc), 2.16 (1H, m, 6α-H), 2.75 (1H, m, 6β-H), 2.92 (1H, m, 5-H), 3.65 (1H, br d, J=9.8 Hz, 9-H), 4.22 (1H, ddd, J=0.5, 8.0, 11.2, 3β-H), 4.33 (1H, ddd, J=3.0, 7.0, 11.2, 3α-H), 4.77 (2H, br s, 10-H_2), 5.85 (1H, br s, 7-H). ^{13}C-NMR ($CDCl_3$, 68 MHz) δ_C: see Table 1. Positive FAB-MS m/z: 211 $(M+H)^+$.

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