Anthraquinones from *Ophiorrhiza hayatana* Ohwi

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Three new naturally occurring anthraquinones, ophiohayatone-A (1), -B (2), and -C (3), together with four known anthraquinones, were isolated from *Ophiorrhiza hayatana* Ohwi (Rubiaceae). Structures of these new compounds were established by spectral methods.

Key words *Ophiorrhiza hayatana*; Rubiaceae; anthraquinone; ophiohayatone

Anthraquinones were a group of plant phenolic products encompassing several hundreds of compounds, differing in the nature and positions of the substituents. They were found in large number of plant families. They were particularly wide spread in the families Rubiaceae, Gesneriaceae and Scrophulariaceae.[1)] Anthraquinones were not only common constituents of plants of the Rubiaceae, but also their tissue and cell cultures. This class of compounds have shown a wide variety of pharmacological activities such as antifungal, antimicrobial, anti-inflammatory, analgesic, antipyretic, antioxtdant and antitumor activities.[2—8)] *Ophiorrhiza*, belongs to the family Rubiaceae, is a small genus of perennial erect herbs distributed in southern Mainland China.[9)] Some species of *Ophiorrhiza* have been used in folk medicine as an antitussive, expectorant, analgesic, and for the treatment of amenorrhea and snakebite.[9)] Members of this genus are well known for their production of alkaloids, in particular camptothecin derivatives and β-carbolines.[10—13)] Although *Ophiorrhiza* belongs to the family Rubiaceae, few reports of anthroquinones were encountered from the hairy roots[12)] and cell cultures.[14)] As a part of our ongoing investigations on the secondary metabolites of Taiwan endemic plants,[15,16)] we have investigated the whole plant of *O. hayatana* Ohwi, a perennial erect herb growing in the low and medium elevations throughout Taiwan.[17)] This paper deals with the isolation and structure elucidation of anthraquinones, named ophiohayatone-A, -B and -C.

Results and Discussion

Ophiohayatone-A (**1**) was obtained as a yellow powder with mp 164—166 °C. The high resolution electron impact mass spectrum (HR-EI-MS) displayed a molecular ion at *m/z* 284.0685 corresponding to the molecular formula $C_{16}H_{12}O_5$. The UV spectrum of **1** showed the absorptions at 214, 278 and 337 nm in MeOH, indicated that **1** was an anthraquinone derivative.[14)] The IR absorption bands at 3407 and 1655 cm^{-1} indicated the presence of hydroxyl and conjugated carbonyl groups. The ^{1}H-NMR spectrum in the aromatic region showed a set of ABX protons at δ 8.04 (1H, d, J=8.6 Hz), 7.48 (1H, d, J=2.4 Hz) and 7.19 (1H, dd, J=8.6, 2.4 Hz) assignable to H-5, H-8 and H-6, respectively of a monosubstituted A-ring of anthraquinone. Two singlets at δ 8.13 and 7.52, attributable to H-4 and H-1, respectively, indicating that **1** was an anthraquinone possessing a 2,3-di-substituted C-ring. The other ^{1}H-NMR signals displayed were a two-proton singlet of oxymethylene group at δ 4.52 and a three-proton singlet of methoxyl group at δ 3.42. The cross-peaks between the carbon of oxymethylene at δ_C 69.1 and a methoxy proton signal at δ_H 3.42 and *vice versa* in the HMBC experiment indicating this group as a methoxymethyl. The HMBC spectrum also showed the correlations between from H-4 (δ_H 8.13) to C-10 (δ_C 181.2) and oxymethylene (δ_C 69.1) which led us to infer the attachment of methoxymethyl group to C-3 of **1**. The signals at 3407, 1655 cm^{-1} in IR and δ_C 184.1 and 181.2 indicated that the two quinone canbonyls were not chelated with the phenol group. Thus, the OH groups have to occupy β-positions (C-2, C-7) of anthraquinone. The up field shift of signals for H-6 and H-1 to δ 7.19 and 7.52, respectively can be attributed to the occurrence of 2,7-dioxygenation in **1**. These results, as well as other correlations observed protons and carbons in 2D NMR, strongly supported the proposed structure of **1** as the new 2,7-dihydroxy-3-methoxymethylanthraquinone.

Ophiohayatone-B (**2**) was isolated as a yellow powder, mp 209—210 °C. The high-resolution FAB-MS displayed a protonated molecular ion at *m/z* 579.1718 corresponding to the molecular formula $C_{27}H_{31}O_{14}$. The UV spectrum of **2** showed absorptions at 246, 253, 266 and 389 nm characteristic of an anthraquinone skeleton. The IR spectrum showed a band for hydroxyl group at 3359 cm^{-1}, in addition to two absorptions for chelated carbonyl at 1629 cm^{-1} and for free carbonyl at 1662 cm^{-1}. This was further confirmed by the two carbonyl down field signals at δ 187.8 and 182.2 in the ^{13}C-NMR spectrum, corresponding to the chelated and nonchelected carbonyl, respectively. The ^{1}H-NMR also supported the chelated nature of the hydroxyl group to the carbonyl by the characteristic downfield signal at δ 13.08 (1H, s). An isolated aryl proton at δ 7.48 and two multiplets of four protons at δ 8.20 and 7.94 in the aromatic region of the ^{1}H-NMR indicated that **2** was 1,2,3-trisubstituted anthraquinone. This was also supported by HMBC correlation between δ_H 7.48 (H-4) and δ_C 182.2 (C-10). A set of oxymethylene protons at δ 4.58 and 4.48 (each 1H, d, J=10.0 Hz) for a hydroxymethyl group were observed in ^{1}H-NMR spectrum. Besides, the presence of two anomeric protons at δ 5.11 (d, J=6.8 Hz) and 4.12 (d, J=7.2 Hz) in ^{1}H-NMR and the carbon signals at δ 101.2, 76.8, 76.4, 73.9, 69.8, 68.7 and 104.8, 77.1, 73.9, 70.2, 66.3 in ^{13}C-NMR suggested the presence of one glucosyl and xylosyl moieties, respectively, with β-configuration. By comparison of these data with the published data of rubiadin 3-*O*-β-primeveroside, the sugar portion was deduced to be primeverosyl moiety (xylosyl-(1→6)-*O*-glucose).[18)] This was supported by downfield shift of the glucosyl C-6′ to δ_C 68.7. To further confirm this proposal, **2** was

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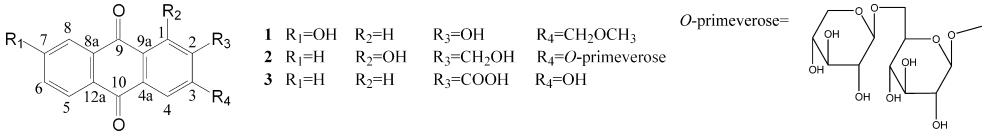

Chart 1

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) Spectral Data of Compounds **1**—**3**

No.	**1**[a]		**2**[b]		**3**[b]	
	δ_H (J=Hz)	δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)	δ_C
1	7.52 s	112.6	—	163.3	8.53 s	131.3
2	—	162.0	—	120.4	—	121.3
3	—	132.6	—	163.0	—	155.8
4	8.13 s	129.9	7.48 s	106.6	7.23 s	115.7
5	8.04 d (8.8)	132.6	8.20 dd (7.6, 2.8)	133.7	8.14 m	135.1
6	7.19 dd (8.8, 1.4)	121.7	7.94 m	135.4	7.85 m	127.2
7	—	163.8	7.94 m	135.6	7.85 m	127.2
8	7.48 d (1.4)	112.8	8.17 dd (7.6, 2.8)	133.5	8.14 m	134.5
9	—	184.1	—	187.8	—	181.3
10	—	181.2	—	182.2	—	183.9
4a	—	134.8	—	127.2	—	137.2
8a	—	125.0	—	127.6	—	134.0
9a	—	135.9	—	111.9	—	124.0
10a	—	181.2	—	135.1	—	134.3
2-$\underline{CH_2}$OH	—		4.58 d (10.0) 4.48 d (10.0)	61.6	—	
3-$\underline{CH_2}OCH_3$	4.52 s	58.2	—		—	
3-$CH_2O\underline{CH_3}$	3.42 s	69.1	3.24 s	58.4	—	
2-COOH	—		—		—	170.1
1′	—		5.11 d (6.8)	101.2	—	
2′	—		3.33 m	73.9	—	
3′	—		3.32 m	76.8	—	
4′	—		3.40 m	69.8	—	
5′	—		3.60 m	76.4	—	
6′	—		3.92 d (9.6) 3.64 m	68.7	—	
1″	—		4.12 d (7.2)	104.8	—	
2″	—		2.98 m	73.9	—	
3″	—		3.06 m	77.1	—	
4″	—		3.39 m	70.2	—	
5″	—		2.97 m 2.68 m	66.3	—	
1-OH	—		13.08 s		—	

a) Solved in acetone-d_6. *b*) Solved in DMSO-d_6.

hydrolyzed with 2 N $HCl_{(aq)}$ to afforded 1,3-dihydroxy-2-hydroxymethyl anthraquinone[19] from an organic layer. The aqueous layer contained D-glucose and D-xylose was proofed by HPLC method. These results supported the primeverosyl moiety substituted on **2**. From ROESY studies, the primeverose residue in **2** was found to be linked to C-3 as NOE of anomeric proton (δ_H 5.11) with the singlet at δ_H 7.48 (H-4) was observed. Since, a hydroxyl group and primerosyl moiety were attached to C-1 and C-3, respectively, the hydroxymethyl group was placed at C-2 of 1,2,3-trisubstituted anthraquinone. On the basis of above data, the structure of **2** was assigned as 1-hydroxy-2-hydroxymethyl anthraquinone-3-*O*-*β*-primeveroside, and named as ophiohayatone-B.

Ophiohayatone-C (**3**) was obtained as yellow powder with mp 191—193 °C. The HR-EI-MS spectrum displayed a molecular ion at m/z 268.0731 corresponding to the molecular formula $C_{15}H_8O_5$. The anthraquinone chromophore was evident from its UV absorption maxima at 220, 246, 259, 279 and 390 nm.[15] The IR absorption bands at 3442 and 1672 cm^{-1} indicated the presence of hydroxyl and conjugated carbonyl groups. The ^{1}H-NMR spectrum in the aromatic region showed two multiplets integrated for four aromatic protons at δ 8.14 and 7.85, and two singlet *peri* protons at δ 8.53 and 7.23, indicating that **3** was 2,3-di-substituted anthraquinone. The ^{13}C-NMR spectrum displaced signals for nonchelated carbonyl groups at δ 183.9 and 181.3 in addition to a signal at δ 170.1 for a carbonylic acid group. Based on the downfield shift of H-1 (δ_H 8.53) and the upfield shift of H-4 (δ_H 7.23) the carboxylic group (δ_C 170.1) and hydroxyl group were placed at C-2 and C-3, respectively. The location of the carboxylic group was also conformed at C-2 due to the ^{3}J correlation between H-1 (δ_H 8.53) and carboxyl carbon at δ_C 170.1 in the HMBC experiment. Thus, **3** was identified as 3-hydroxyanthraquinone-2-carboxylic acid. This compound had synthesised by Hayashi in 1953.[20] However, this is the first report of **3** as a natural product.

In addition, four anthraquinones, rubiadin,[21] rubiadin-3-*O*-*β*-

primeveroside,[18] 2-hydroxymethyl-3-methoxyanthraquinone[12] and 1-hydroxy-2-hydroxymethylanthraquinone-3-*O*-β-glucoside[22] were also isolated from the methanol extract of *O. hayatana*. All these known compounds were identified by comparison of their spectroscopic data (UV, IR, NMR, MS spectrometry) with the authentic samples or literature data.

Kitajima *et al.* reported several anthraquinones from *O. pumila* tissue and cell cultures and found a remarkable difference in the constituents between the wild plants and the callus tissue or cultured cells; the former was devoid of anthraquinones and contained a variety of camptothecin-related alkaloids whereas the latter contained a significant amount of anthraquinones but no indication of alkaloids.[14] However, the present study revealed the presence of eight anthraquinones in the wild plants of *O. hayatana* collected in Taiwan. All the anthraquinones identified from *Ophiorrhiza* so far were only substituted in the C-ring, except ophiohayatone-A (**1**) from the *O. hayatana*, with monosubstituted A-ring. This is remarkable, because in the family Rubiaceae the anthraquinones were considered to be derived from shikimate and mevalonate and originally it was considered to be a characteristic of these anthraquinones that they were substituted in only one ring.[23,24] This led us to question the presumed biosynthesis of anthraquinones in *Ophiorrhiza*. These results might indicate a different pathway for anthraquinone formation with in the family Rubiaceae, as Schripscma *et al.* concluded from the studies on *Cinchona*.[23] Further, it has to be pointed out that only a limited number of experiments have been performed to determine the biosynthetic route to the anthraquinones in Rubiaceae, of which none have been performed on *Ophiorrhiza*. Only a more thorough investigation of anthraquinone biosynthesis with in Rubiaceae will yield the definitive conclusions.

Experimental

General Procedure Melting points were measured on a Yanagimoto MP-S3 micro melting point apparatus and are uncorrected. The UV spectra were recorded on a Hitachi UV-3210 spectrophotometer in MeOH solution. The IR spectra were measured on a Shimadzu FTIR-8501 spectrophotometer as KBr disks. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Varian-400 Unity Plus spectrometer. Chemical shifts are shown in δ values with tetramethylsilane as an internal reference. The Mass spectra (EI or FAB) were performed on a VG 70-250 S spectrometer. Determination of free carbohydrates was performed by high performance liquid chromatography (HPLC) on a Shimadzu LC-10AT*VP* series system equipped with a Shimadzu RID 10A refractive-index detector, and a Merck Lichrosorb® NH_2 column (5 mm). The standard sugars (D-glucose and D-xylose) were purchased from Acros Organics (Belgium).

Plant Material *Ophiorrhiza hayatana* Ohwi was collected from Tainan, Taiwan in March 2003 and verified by Prof. C. S. Kuoh. A voucher specimen was deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Separation The air-dried and powdered whole plant of *O. hayatana* Ohwi (19.5 kg) was extracted with hot methanol (5 l×6) and concentrated. The concentrated extract was partitioned with water and chloroform and the resulted chloroform layer (285 g) was chromatographed over silica gel using a gradient of chloroform and methanol to afford 12 fractions. Fraction 3 was rechromatographed over silica gel using mixture of diisopropylether and methanol (9 : 1) as eluents, and purified by recrystallation and preparative TLC to yield **1** (4.2 mg), rubiadin (0.02 mg) and 1-hydroxy-2-hydroxymethylanthraquinone-3-*O*-β-glucoside (0.15 mg). Fraction 10 on column chromatography with chloroform and methanol (4 : 1) yielded **3** (1.8 mg).

The water-soluble fraction (72 g) was chromatographed over Diaion HP-20 using water–methanol gradients, which yielded 13 fractions. Fraction 12 was column chromatographed with silica gel using mixture chloroform and methanol (4 : 1) afforded rubiadin-3-*O*-β-primeveroside (0.25 mg). The *t*-buthanol-soluble fraction (90 g) was chromatographed over Diaion HP-20 using water–methanol gradients, which yielded 10 fractions. Fraction 8 was column chromatographed with silica gel using gradients of chloroform, ethyl acetate and methanol (2 : 2 : 1) afforded **2** (85.2 mg).

Ophiohayatone-A (**1**): Yellow powder, mp: 164—166 °C. HR-EI-MS *m/z* 284.0685 (Calcd for $C_{16}H_{12}O_5$, 284.0685). UV λ_{max} (MeOH) nm (log ε): 214 (4.3), 278 (4.7), 337 (3.7); IR ν_{max} (KBr) cm^{-1}: 3407, 1655, 1579; EI-MS *m/z* (rel. int.): 284 (M^+, 71), 254 (26), 252 (100), 224 (38), 196 (75), 139 (42); ^{1}H-NMR (400 MHz, acetone-d_6) δ: 8.13 (1H, s, H-4), 8.04 (1H, d, J=8.6 Hz, H-5), 7.52 (1H, s, H-1), 7.48 (1H, d, J=2.4 Hz, H-8), 7.19 (1H, dd, J=8.6, 2.4 Hz, H-6), 4.52 (2H, s, CH_2), 3.42 (3H, s, OCH_3); ^{13}C-NMR (100 MHz, acetone-d_6) δ: 184.1 (C-9), 181.2 (C-10), 163.8 (C-7), 162.0 (C-2), 135.9 (C-9a), 134.8 (C-4a), 132.6 (C-3), 132.6 (C-5), 129.9 (C-4), 125.8 (C-10a), 125.0 (C-8a), 121.7 (C-6), 112.8 (C-8), 112.6 (C-1), 69.1 (C–OCH_3), 58.2 (C–CH_2).

Ophiohayatone-B (**2**): Yellow powder, mp: 209—210 °C. $[\alpha]_D$ −50.8° (c=0.024, MeOH); HR-FAB-MS *m/z* 579.1718 (Calcd for $C_{27}H_{31}O_{14}$, 579.1715). UV λ_{max} (MeOH) nm (log ε): 246 (4.5), 253 (4.4), 266 (4.6), 389(3.9); IR ν_{max} (KBr) cm^{-1}: 3359, 1662, 1629, 1591; FAB-MS *m/z* (rel. int.): 579 ($[M+H]^+$, 1), 369 (14), 333 (14), 303 (21), 185 (78), 93 (100); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.08 (1H, s, 1-OH), 8.20 (1H, dd, J=7.6, 2.8 Hz, H-5), 8.17 (1H, dd, J=7.6, 2.8 Hz, H-8), 7.94 (2H, m, H-6, H-7), 7.48 (1H, s, H-4), 5.11 (1H, d, J=6.8 Hz, H-1′), 4.58 (1H, d, J=10.0 Hz, CH_2-b), 4.48 (1H, d, J=10.0 Hz, CH_2-a), 4.12 (1H, d, J=7.2 Hz, H-1″), 3.92 (1H, d, J=9.6 Hz, H-6′b), 3.68 (1H, m, H-5″b), 3.64 (1H, m, H-6′a), 3.60 (1H, m, H-5′), 3.40 (1H, m, H-4′), 3.39 (1H, m, H-4″), 3.33 (1H, m, H-2′), 3.32 (1H, m, H-3′), 3.24 (3H, s. OCH_3), 3.06 (1H, m, H-3″), 2.98 (1H, m, H-2″), 2.97 (1H, m, H-5″a); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 187.8 (C-9), 182.2 (C-10), 163.3 (C-1), 163.0 (C-3), 135.6 (C-7), 135.4 (C-6), 135.1 (C-10a), 133.7 (C-5), 133.5(C-8), 127.6 (C-8a), 127.2 (C-4a), 120.4 (C-2), 111.9 (C-9a), 106.6 (C-4), 104.8 (C-1″), 101.2 (C-1′), 77.1 (C-3″), 76.8 (C-3′), 76.4 (C-5′), 73.9 (C-2″), 73.9 (C-2′), 70.2 (C-4″), 69.8 (C-4′), 68.7 (C-6′), 66.3 (C-5″), 61.6 (C–CH_2), 58.4 (C–OCH_3).

Hydrolysis of Ophiohayatone-B (2) and Detection of the Sugar Moiety **2** (5 mg) was dissolved in 5 ml 2 N $HCl_{(aq)}$ for 6 h at 50 °C. Then, H_2O and EtOAc (each 10 ml) were added, and the EtOAc layer was washed with H_2O and then evaporated to dryness. Recrystallization of this residue gave 1,3-dihydroxy-2-hydroxymethyl anthraquinone (2.0 mg). The H_2O layer was neutralized by 0.5 N $NaOH_{(aq)}$ and analyzed by HPLC with an acetonitrile and water eluent (83 : 17, flowing rate=0.9 ml/min). The retention times of soluble D-glucose and D-xylose standards were compared with those of the sample (retention time of D-glucose: 17.2 min; retention time of D-xylose: 12.6 min).

Ophiohayatone-C (**3**): Yellow powder, mp: 191—193 °C. HR-EI-MS *m/z* 268.0731 (Calcd for $C_{15}H_8O_5$, 268.0372). UV λ_{max} (MeOH) nm (log ε): 220 (3.8), 246 (4.0), 259 (3.8), 279 (4.1), 390 (3.1); IR ν_{max}(KBr) cm^{-1}: 3442, 1672, 1634, 1589, 1576; EI-MS *m/z* (rel. int.): 268 (M^+, 18), 249 (39), 207 (23), 149 (29), 131 (85), 129 (100), 88 (51), 69 (91); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.53 (1H, s, H-1), 8.14 (2H, m, H-5, H-8), 7.85 (2H, m, H-6, H-7), 7.23 (1H, s, H-4); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 183.9 (C-10), 181.3 (C-9), 170.1 (C–COOH), 155.8 (C-3), 137.2 (C-4a), 135.1 (C-5), 134.5 (C-8), 134.3 (C-10a), 134.0 (C-8a), 131.3 (C-1), 127.2 (C-7), 127.2 (C-6), 124.0 (C-9a), 121.3 (C-2), 115.7 (C-4).

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