Studies on Constituents of *Evodia rutaecarpa* (Rutaceae). I. Constituents of the Leaves[1)]

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A new glycoside, evodioside B (1) was isolated from the *n*-BuOH-soluble fraction of the MeOH extract of the leaves of *Evodia rutaecarpa* (JUSS.) BENTH. (Rutaceae) together with rutin and hyperin. Epimedoside C (2), hyperin, and guaijaverin were isolated from the EtOAc-soluble fraction and dehydroevodiamine·hydrogenchloride (3) was isolated from the $CHCl_3$-soluble fraction as were limonin and β-sitosterol. The structure of compound 1 was determined to be 5,7,4′-trihydroxy-8-isopentenylflavanone 7,4′-di-*O*-β-D-glucopyranoside on the basis of spectroscopic evidence, and the ^1H- and ^{13}C-NMR signals of 2 and 3 were completely assigned.

Keywords evodioside B; dehydroevodiamine·hydrogenchloride; *Evodia rutaecarpa*; epimedoside C; guaijaverin; Rutaceae

Evodia rutaecarpa BENTHAM (Rutaceae) is an original plant of several crude drugs, Evodia fruit (Goshuyu), Evodia leaf (Goshuyu-yo), and Evodia root (Goshuyu-kon). The Evodia fruit has been used in traditional Chinese prescriptions and there are many studies on the Evodia fruit and its prescriptions. However, reports on the leaves and roots are rate. Therefore, we have examined the constituents of the leaves and the roots of *E. rutaecarpa*. Previously, Nakasato *et al.* reported the isolation of hydroxyevodiamine and choline as alkaloid constituents[2)] of the leaves. Bodalski *et al.* isolated an unidentified flavanone glucoside from the same source,[3)] and its structure has been reported by Grimshaw and Lamer-Zarawska to be an isopentenylflavanone, except for the location of the isopentenyl group.[4)]

In this work, we examined the constituents of the fresh leaves of this plant and as result, a new glycoside of prenylated flavonoid, named evodioside B (**1**) and dehydroevodiamine·hydrogenchloride (**3**) were isolated, together with epimedoside C (**2**), rutin, hyperin, guaijaverin, limonin, and β-sitosterol. This paper deals with the structure elucidation of **1** and **3**, and the identification of **2**, including the complete assignment of the ^1H- and ^{13}C-NMR spectra of **1**, **2**, and **3**.

Results and Discussion

The MeOH extract of the fresh leaves of *E. rutaecarpa* was successively extracted with $CHCl_3$, EtOAc, and *n*-BuOH. A Sephadex LH-20 column chromatography of the *n*-BuOH-soluble fraction gave a new isopentenylflavanone glucoside named evodioside B (**1**), together with rutin and hyperin. The EtOAc-soluble fraction was also chromatographed on a Sephadex LH-20 column to afford hyperin, guaijaverin, and an isopentenylflavonol glucoside, epimedoside C (**2**). The $CHCl_3$ extract was chromatographed on a silica gel column to afford β-sitosterol, limonin, and dehydroevodiamine·hydrogenchloride (**3**).

Evodioside B (**1**) exhibited positive $FeCl_3$ reaction and positive color reaction for flavonoids. The UV spectrum of **1** in MeOH solution exhibited absorptions at 287 and 341 nm, which are typical spectra for flavanone or dihydroflavonol,[5)] and with the addition of $AlCl_3$/HCl to the solution, a significant bathochromic shift of band II was observed, while no shifts were observed following the addition of NaOAc or NaOMe. These UV spectral data suggested the presence of a hydroxyl group at the 5-position of the flavanone skeleton.[5)] The ^1H-NMR spectrum of **1** showed a hydroxyl proton signal at δ 12.07 ppm, a pair of 2H doublet (J=8.8 Hz) at δ 7.44 and 7.05 ppm assignable to H-2′,6′ and H-3′,5′, a signal for an isolated aromatic proton at δ 6.27 ppm, a methine proton signal (dd, J=3.1, 12.2 Hz), at δ 5.53 ppm assignable to H-2, single-proton methylene signals at δ 2.85 (2H, dd, J=3.1, 17.2 Hz) and 3.22 (m) ppm assignable to H-3, and two vinyl methyl signals at δ 1.60 and 1.59 ppm. Two signals at δ 4.91 and 4.90 ppm (1H, each d, J=7.3 Hz) were assignable to the anomeric protons of two sugar moieties in the β-configuration.

The ^{13}C-NMR spectrum of **1** exhibited twenty-nine signals, including three overlapping ones. Those at δ 197.1 (s), 163.1 (s), 161.2 (s), 158.7 (s), 157.4 (s), 132.0 (s), 127.6 (d × 2), 116.1 (d × 2), 108.9 (s), 103.1 (s), 95.1 (d), 78.0 (d), and 42.3 ppm (t) suggested the presence of a 5,7,4′-trioxygenated-6- or 8-*C*-substituted flavanone skeleton,[6)] and five signals at δ 130.2 (s), 122.6 (d), 25.5 (q), 21.4 (t), and 17.6 ppm (q) were assignable to an isopentenyl group.[5)] The other ^{13}C-signals at δ 100.3 and 100.2 (d), 77.1 and 77.0 (d), 76.6 (d × 2), 73.2 and 73.1 (d), 69.63 and 69.57 (d), and 60.7 and 61.0 ppm (t) were assignable to two β-D-glucopyranosyl moieties.[6)] From the above evidence, **1** was assumed to be 6- or 8-isopentenylflavanone 5,7,4′-trihydroxy-7, 4′-di-*O*-β-D-glucopyranoside.

To clarify the structure of **1**, we applied two-dimensional (2D)-NMR techniques such as proton–proton (H–H), carbon–proton (C–H), and long range C–H (LRCH) shift correlation spectroscopy (COSY) and difference nuclear Overhauser effect (NOE) spectra. The assignments of ^1H- and ^{13}C-signals determined by the H–H, C–H, and LRCH methods are shown in Table I.

The location of an isopentenyl group was determined to be at the 8-position of the flavanone skeleton according to the following evidence. The ^1H-signals at δ 12.07 ppm (5-OH) showed significant long-range correlation with the ^{13}C-signals at δ 103.1 (s, C-10), 161.2 (s, C-5), and 95.1 (d) ppm; therefore, the signal at δ 95.1 ppm was assigned to C-6. Subsequently, the ^1H-signal at δ 6.27 ppm, assignable

TABLE I. Assignments of ^1H- and ^{13}C-signals of Evodioside B (**1**)

Positions	^1H	^{13}C	Positions	^1H	^{13}C
2	5.53 (1H, dd)	78.0 (d)	G-1	4.91 (1H, d)	100.2 (d)
3	3.22 (1H, m)	42.3 (t)		4.90 (1H, d)	100.3 (d)
	2.85 (1H, dd)		G-2	3.29 (2H, m)	73.2 (d)
4		197.1 (s)			73.1 (d)
5		161.2 (s)	G-3	3.29 (2H, m)	76.6 (d, ×2)
6	6.27 (1H, s)	95.1 (d)	G-4	3.19 (2H, m)	69.63 (d)
7		163.1 (s)			69.57 (d)
8		108.9 (s)	G-5	3.38 (2H, m)	77.1 (d)
9		157.4 (s)			77.0 (d)
10		103.1 (s)	G-6	3.72 (2H, m)	60.7 (t)
11	3.34 (1H, m)	21.4 (t)		3.50 (2H, m)	61.0 (t)
	3.09 (1H, dd)		5-OH	12.07 (1H, s)	
12	5.16 (1H, m)	122.6 (d)	G-2-OH	5.31 (1H, m)	
13		130.2 (s)		5.29 (1H, m)	
14	1.59 (3H, s)	17.6 (q)	G-3-OH	5.14 (2H, m)	
15	1.60 (3H, s)	25.5 (q)	G-4-OH	5.04 (2H, m)	
1′		132.0 (s)	G-6-OH	4.60 (1H, m)	
2′, 6′	7.44 (2H, d)	127.6 (d, ×2)		4.54 (1H, m)	
3′, 5′	7.08 (2H, d)	116.1 (d, ×2)			
4′		158.7 (s)			

to H-6 by the C–H COSY method, showed significant long-range correlation with the ^{13}C-signals at δ 103.1 (C-10), 108.9 (s), 161.2 (C-5), and 163.1 ppm (s, C-7). Therefore, the quaternary carbon signal at δ 108.9 ppm was assignable to C-8, and consequently, the isopentenyl group was located at the 8-position.

In the difference NOE spectra of **1**, negative NOEs were mutually observed between the anomeric proton signals at δ 4.90 and 4.91 ppm and between the aromatic proton signals at δ 6.27 and 7.44 ppm; therefore, two β-D-glucopyranosyls were located at the 7-*O*- and 4′-*O*-positions.

Thus evodioside B (**1**) is considered to be 5,7,4′-trihydroxy-8-isopentenylflavanone 7,4′-di-*O*-β-D-glucopyranoside. The compound shows a Cotton effect in its optical rotatory dispersion (ORD)-curve and a comparison of its ORD-curves with those of naringin and hesperidin[7] indicates the 2*S*-configuration for evodioside B (**1**).

Compound **2** showed a positive $FeCl_3$ reaction and a positive color reaction for a flavonoid. The spot color of **2** in UV light (UVL) appeared to be yellow, suggesting the presence of a hydroxyl group at the 3-position of the flavonoid.[8] The UV spectrum of **2** in MeOH solution exhibited absorptions at 253 (sh), 270, 327, and 374 nm, and with each addition of $AlCl_3$/HCl and NaOMe to the solution, significant bathochromic shifts were observed. These UV spectral data suggested the presence of hydroxyl groups at the 5- and 4′-positions of a flavonol skeleton.[9] The ^1H-NMR spectrum of **2** showed signals due to three hydroxyl protons, a pair of *ortho*-coupled aromatic protons, an isolated aromatic proton, an olefinic proton, two vinyl methyls, and an anomeric proton for the β-configuration of a sugar moiety. The ^{13}C-NMR spectrum of **2** exhibited twenty-four signals including two overlapping ones, and its spectral pattern suggested the presence of either a 3,5,7,4′-tetraoxygenated-6- or 8-*C*-substituted flavone skeleton, an isopentenyl group, and a β-D-glucopyranosyl moiety.[6] From the above evidence, **2** was assumed to be 3,5,7,4′-tetrahydroxy-6- or -8-isopentenylflavone 7-*O*-β-D-glucopyranoside. The complete assignment of ^1H- and ^{13}C-signals was determined by C–H, LRCH and LSPD methods. The location of an isopentenyl group and a β-D-glucopyranosyl moiety of **2** was determined, respectively, to be at the 8- and 7-*O*-positions of the flavone skeleton using by the same method described for **1**. From the foregoing evidence, compound **2** was identified as 3,5,7,4′-tetrahydroxy-8-isopentenylflavone-7-*O*-β-D-glucopyranoside, that is, epimedoside C.[10,11]

Compound **3** showed a positive reaction with Dragendorff reagent. The IR spectrum of **3** showed absorption bands at 3440 (NH), 1700 (CO), and 1610 cm^{-1} (benzene ring). The UV spectrum of **3** in MeOH solution exhibited absorptions at 246, 313, and 365 nm, which is similar to that of dehydroevodiamine.[2] In the energy dispersive X-ray (EDX) microanalysis of **3**, the X-ray spectrum showed a peak at 2.63 keV which is corresponded to the energy position of Cl ($K_{\alpha 1,2}$). Sublimation of compound **3** *in vacuo* gave a crystalline compound which was identified as rutaecarpine (**4**). The ^1H-NMR spectrum of **3** showed signals due to a secondary amine (NH) at δ 12.70 ppm which disappeared with the addition of D_2O, two 1,2-disubstituted benzene rings at δ 8.35, 8.19, 8.13, 7.88, 7.80, 7.72, 7.52, and 7.24 ppm, two methylene protons at δ 4.47 and 3.33 ppm, and a methyl at δ 4.40 ppm. The ^{13}C-NMR spectrum of **3** exhibited 19 signals due to a methyl at δ 40.8 ppm, a carbonyl at δ 158.2 ppm, two methylenes at δ 18.5 and 42.0 ppm, and 15 aromatic carbons at δ 149.9 (s), 141.4 (s), 139.6 (s), 136.7 (d), 130.4 (s), 128.8 (d), 128.6 (d), 127.7 (d), 123.3 (s), 121.61 (d), 121.5 (d), 120.0 (s), 118.7 (s), 118.4 (d), and 113.5 ppm (d). Assignment of ^1H- and ^{13}C-signals completed by the techniques of H–H decoupling, difference NOEs, C–H, LRCH, and heteronuclear multiple bond connectivity (HMBC) methods are shown in the Experimental section. Dehydroevodiamine was obtained from a C_6H_6 solution of hydroxyevodiamine and it formed a crystalline salt with hydrochloride.[2] Compound **3** corresponds to a dehydroevodiamine·hydrogenchloride on the basis of the complete assignment of ^1H- and ^{13}C-NMR spectra and EDX microanalysis of **3**. This is the first report on the isolation of **3** from natural sources. However, it is undeniable that **3** is formed by the extraction with $CHCl_3$ as an artifact.

The other known compounds, rutin, hyperin, guaijaverin, β-sitosterol, and limonin, were identified by direct comparison with the authentic samples and by spectral comparison with those of published data. The constituents of the MeOH extract of the roots of *E. rutaecarpa* are now under investigation.

Experimental

General Procedures All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. UV and IR spectra were recorded respectively on a Hitachi 220 S double beam spectrophotometer and a 260-10 infrared spectrometer with polystyrene calibration at 1601 cm^{-1}. Specific rotation and ORD were measured on a JASCO DIP-400 digital polarimeter and JASCO J-20 ORD meter, respectively. ^1H- and ^{13}C-NMR spectra were taken on a JEOL JNM-GX 270 or 400 spectrometer at 270 or 400 and 67.9 or 100 MHz, respectively, with tetramethylsilane as an internal standard. The chemical shifts are recorded in δ (ppm) values. Multiplicities of ^{13}C-NMR data were determined by the distortionless enhancement by polarization transfer (DEPT) method and are indicated as s, d, t, and q. 2D-NMR spectra were taken on a JEOL JNM-GX-400 spectrometer. EI- and HREI-MS and positive ion FAB-MS were obtained on a JEOL JMS-D 200 (operating at 70 eV) and JMS-DX 300 mass spectrometer. The EDX microanalysis was employed on a Hitachi Scanning Electron Microanalyzer X-650 equipped with a Kevex 700 Q EDX spectrometer, accelerating voltage: 20 kV, electron beam current; 0.1×10^{-9} A, analyzing time: 100 s.

Plant Materials The leaves of *E. rutaecarpa* grown in our herbal garden were collected in June, 1991. The voucher specimen has been deposited in the herbarium of our university.

Extraction and Isolation The fresh leaves of *E. rutaecarpa* (3 kg) were thrice extracted with MeOH at room temperature for 3 d. The MeOH solution was evaporated to give the MeOH extract (298 g). The extract was extracted successively with $CHCl_3$, EtOAc, and *n*-BuOH, and individual extracts were obtained in yields of 79, 11, and 43 g, respectively. The *n*-BuOH-soluble fraction was chromatographed on a Sephadex LH-20 column by elution with 50% MeOH to give rutin (530 mg), **1** (50 mg), and hyperin (1.4 g). The EtOAc extract was treated with 50% MeOH to give hyperin (1.2 g) and the filtrate was chromatographed on a Sephadex LH-20 column as described for the *n*-BuOH-soluble fraction to give **2** (65 mg), hyperin (5 mg) and guaijaverin (40 mg). The $CHCl_3$ extract was chromatographed on a silica gel column by elution with a mixture of MeOH/$CHCl_3$ to give β-sitosterol (330 mg), limonin (1087 mg), and **3** (235 mg).

Evodioside B (1) Colorless micro needles, mp 245—247 °C. $[\alpha]_D^{20}$ +41° (c=1.0, pyridine). Purplish brown with $FeCl_3$, red with Mg+HCl, and pink with Zn+HCl. Dark brown under UVL. UV λ_{max}^{MeOH} nm (log ε): 287 (4.38), 341 (3.99); $\lambda_{max}^{MeOH+AlCl_3}$ nm: 312, 397; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 310, 393; $\lambda_{max}^{MeOH+NaOAc\ and\ MeOH+NaOAc+H_3BO_3}$ nm similar to λ_{max}^{MeOH}; $\lambda_{max}^{MeOH+NaOMe}$ nm: 287, 353. IR ν_{max}^{KBr} cm^{-1}: 3400, 1640, 1590, 1515, 1380, 1300, 1250, 1230, 1180, 1075, 1030, 835. Positive FAB-MS m/z: 665 (M+H), 503 (M+H−$C_6H_{10}O_5$). ORD (c=0.3, EtOH) $[\phi]_{298}$ −86300, $[\phi]_{273}$ +54400. ^{1}H- and ^{13}C-NMR (DMSO-d_6) see Table I.

Epimedoside C (2) Yellow micro needles, mp 289 °C. Purplish brown with $FeCl_3$, orange with Mg+HCl, and pink with Zn+HCl. Yellow under UVL. UV λ_{max}^{MeOH} nm (log ε): 253 (sh), (4.29), 270 (4.35), 327 (4.13); $\lambda_{max}^{MeOH+AlCl_3\ and\ MeOH+AlCl_3+HCl}$ nm: 267, 357, 433; $\lambda_{max}^{MeOH+NaOAc}$ nm: 253 (sh), 270, 327, 383; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm similar to λ_{max}^{MeOH}; $\lambda_{max}^{MeOH+NaOMe}$ nm: 249, 267 (sh), 418. IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1610, 1600, 1560, 1540, 1510, 1420, 1350, 1180, 1090, 1070, 1040, 995, 835, 800. Positive FAB-MS: m/z: 517 (M+H), 355 (M+H−$C_6H_{10}O_5$). EI-MS m/z: 354 (M−$C_6H_{10}O_5$), 340, 300, 287, 203, 165, 121. ^{1}H-NMR (DMSO-d_6) δ: 12.47 (1H, s, 5-OH, disappeared with D_2O), 10.12 (1H, s, 4′-OH, disappeared with D_2O), 9.42 (1H, s, 3-OH, disappeared with D_2O), 8.07 (2H, d, J=8.5 Hz, 2′,6′-H), 6.95 (2H, d, J=8.5 Hz, 3′,5′-H), 6.60 (1H, s, 6-H), 5.22 (1H, m, 12-H), 5.02 (1H, d, J=7.6 Hz, glucosyl anomer H), 3.66 (1H, m, 11-H_a), 3.45 (1H, m, 11-H_b), 1.78 (3H, s, 15-H_3), 1.64 (3H, s, 14-H_3). ^{13}C-NMR (DMSO-d_6) δ 176.3 (s, C-4), 160.0 (s, C-7), 159.3 (s, C-4′), 158.6 (s, C-5), 152.7 (s, C-9), 147.4 (s, C-2), 135.8 (s, C-3), 130.9 (s, C-13), 129.5 (d, C-2′, C-6′), 122.4 (d, C-12), 121.8 (s, C-1′), 115.4 (d, C-3′, C-5′), 108.0 (s, C-8), 104.4 (s, C-10), 100.5 (d, G-1), 97.4 (d, C-6), 77.2 (d, G-5), 76.6 (d, G-3), 73.4 (d, G-2), 69.7 (d, G-4), 60.7 (d, G-6), 25.5 (q, C-15), 21.4 (t, C-11), 18.0 (q, C-14).

Dehydroevodiamine·Hydrogenchloride (3) Yellow micro needles, mp 215—218 °C. A positive reaction with Dragendorff reagent. UV λ_{max}^{MeOH} nm (log ε): 246 (4.51), 313 (4.02), 365 (4.71). IR ν_{max}^{KBr} cm^{-1}: 3440, 1700, 1610, 1550, 1430, 1380, 1340, 1280, 1260, 1235, 1210, 1105, 760, 750, 735. EI-MS m/z: 302 (M−Cl), 301 (M−HCl), 287, 286, 165, 156, 144. HREI-MS m/z: 302.1283, Calcd for $C_{19}H_{16}N_3O$, 302.1293; m/z: 301.1201, Calcd for $C_{19}H_{15}N_3O$, 301.1215. ^{1}H-NMR (DMSO-d_6) δ: 12.70 (1H, s, 1-H, disappeared with D_2O), 8.35 (1H, dd, J=1.2, 8.5 Hz, 19-H), 8.19 (1H, d, J=8.5 Hz, 16-H), 8.13 (1H, dt, J=1.2, 8.5 Hz, 17-H), 7.88 (1H, d, J=8.5 Hz, 9-H), 7.80 (1H, dt, J=1.2, 8.5 Hz, 18-H), 7.72 (1H, d, J=8.5 Hz, 12-H), 7.52 (1H, dt, J=1.2, 8.5 Hz, 11-H), 7.27 (1H, t, J=8.5 Hz, 10-H), 4.47 (2H, t, J=6.7 Hz, 5-H_2), 4.40 (3H, s, CH_3), 3.33 (2H, t, J=6.7 Hz, 6-H_2). ^{13}C-NMR (DMSO-d_6) δ: 158.2 (s, C-21), 149.9 (s, C-3), 141.4 (s, C-13), 139.6 (s, C-15), 136.7 (d, C-17), 130.4 (s, C-7), 128.8 (d, C-11), 128.6 (d, C-18), 127.7 (d, C-19), 123.3 (s, C-8), 121.6 (d, C-10), 121.5 (d, C-9), 120.0 (s, C-2), 118.7 (s, C-20), 118.4 (d, C-16), 113.5 (d, C-12), 42.0 (t, C-5), 40.8 (q, C-22), 18.5 (t, C-6).

EDX Microanalysis of 3 The sample of **3**, which was saved on an aluminium plate coated with carbon powder, was first viewed under a transmission electron microscope and then examined under a scanning transmission electron microscope as an image. The peak was detected at 2.63 keV which corresponds to the energy position of the $K_{\alpha 1,2}$ of Cl.

Sublimation of 3 Compound **3** was sublimated on an oil bath *in vacuo* to give cololress needles, mp 255—257 °C. HREI-MS m/z: 287.1057, Calcd for $C_{18}H_{13}N_3O$, 287.1058. It was identified by direct comparison with an authentic rutaecarpine (**4**) isolated from the fruit of *E. rutaecarpa*.

Identification of Known Compounds Rutin, yellow needles, mp 185—189 °C, hyperin, yellow needles, mp 233—235 °C, β-sitosterol, colorless needles, mp 143 °C, and limonin, colorless needles, mp 257—259 °C, were identified by direct comparison with authentic samples. Guaijaverin, yellow micro needles, mp 190—192 °C, was identified by a comparison of the spectral data with published values.[6]

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