Notes

A New Indolopyridoquinazoline-Type Alkaloid from *Phellodendron amurense* Callus Tissues

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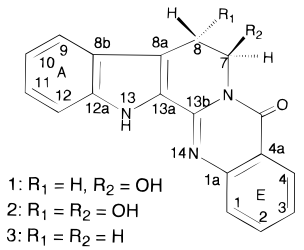
Callus tissue from the stems of *Phellodendron amurense* (Rutaceae) produced a new indolopyridoquinazoline-type alkaloid, 7, 8-dihydroxyrutaecarpine (**2**), together with known 7-hydroxyrutaecarpine (**1**). Their structures were established using spectroscopic methods.

The Chinese crude drug, the bark of *Phellodendron amurense* Rupr. (amur cork tree, Rutaceae), has been used as a stomachic for intestinal function control and as an antibacterial and an antiinflammatory agent. The major chemical constituents of its bark are isoquinoline alkaloids, such as berberine.[1] Phenolic compounds and flavone glucosides have also been isolated from the bark[2] and the leaves,[3] respectively, together with phytosterols and limonoidal triterpenes.[4] We reported previously the establishment of a callus tissue line from the stems of *P. amurense*. Callus tissues produced indolopyridoquinazoline-type alkaloids; rutaecarpine, 7,8-dehydrorutaecarpine; canthinone-type, canthin-6-one, and furoquinoline-type alkaloids, dictamnine, γ-fagarine and skimmianine, as the main alkaloid components, along with small amounts of isoquinoline-type alkaloids (berberine, palmatine, and magnoflorine).[5] The indolopyridoquinazoline-, canthinone-, and furoquinoline-type alkaloids have not been reported from *P. amurense*, although canthin-6-one and berberine were reported from the root and the stems of the young plant. We now report the isolation and the structure elucidation of a new indolopyridoquinazoline-type alkaloid, 7,8-dihydroxyrutaecarpine (**2**).



Callus was established from the stems of *P. amurense* on Murashige and Skoog[7] medium containing 2,4-D (3 mg/L) with Kinetin (0.1 mg/L) as plant growth regulators. The stock callus tissues (130 g, dry weight) were subcultured during 8 months at 5-week intervals and were extracted successively with MeOH and EtOAc. Both extracts were combined and subjected repeatedly to chromatography on a silica gel column, with gradient elution using hexane and increasing the proportion of EtOAc, to give two indolopyridoquinazoline-type alkaloids (**1** and **2**).

Table 1. ^{1}H NMR Data (δ) for Compounds **1** and **2**

no.	compd **1**[a]	compd **2**[b]
H-1	7.58 (1H, d J = 8 Hz)	7.74 (1H, dd, J = 8, 1 Hz)
H-2	7.69 (1H, ddd, J = 8, 7.5, 1.5 Hz)	7.80 (1H, ddd, J = 8.5, 7.5, 1.5 Hz)
H-3	7.35 (1H, dd, J = 8, 7.5 Hz)	7.48 (1H, ddd, J = 8, 7.5, 1 Hz)
H-4	8.21 (1H, dd, J = 8, 1.5 Hz)	8.24 (1H, dd, J = 8, 1.5 Hz)
H-7	6.88 (1H, dd, J = 5.5, 1.5 Hz)	6.70 (2H, d, J = 2 Hz)
H-8	3.42 (1H, dd, J = 17, 5.5 Hz, H-8ax) 3.59 (1H, dd, J = 17, 1.5 Hz, H-8eq)	5.24 (2H, d, J = 2 Hz)
H-9	7.60 (1H, d, J = 7.5 Hz)	7.76 (1H, dd, J = 8, 1 Hz)
H-10	7.15 (1H, ddd, J = 8, 7, 1 Hz)	7.16 (1H, ddd, J = 8.5, 7.5, 1 Hz)
H-11	7.31 (1H, ddd, J = 8.5, 7, 1 Hz)	7.29 (1H, ddd, J = 8.5, 7.5, 1 Hz)
H-12	7.42 (1H, d, J = 8.5 Hz)	7.51 (1H, dd, J = 8, 1 Hz)

[a] Measured in $CDCl_3$. [b] Measured in CD_3OD.

Compound **1** was isolated as a colorless powder. The UV spectrum showed the presence of a highly conjugated system with characteristic absorption bands for indolopyridoquinazoline-type alkaloids.[8] The IR spectrum exhibited maxima at 1647, 1595, and 1553 cm^{-1}. The mass spectrum showed a M^+ at m/z 303 and 285 [M − H_2O]. In the aromatic region of the ^{1}H NMR spectrum (CD_3OD, δ, ppm), two pairs of four-mutually coupled protons (Table 1) were characteristic of ortho-disubstituted benzenoid rings. These data suggested that **1** is an indolopyridoquinazoline-type alkaloid very similar to rutaecarpine (**3**).[5] On the basis of the above data and comparison of ^{13}C NMR data with literature values (Table 2), compound **1** was identified as 7-hydroxyrutaecarpine, reported previously from *Evodia meliafolia*.[9]

Compound **2** also gave a UV spectrum (λ_{max}, EtOH) indicative of a highly conjugated system (λ_{max} 215, 240

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Table 2. ^{13}C NMR Data (δ) for Compounds **1** and **2**

no.	**1**[a]	**1**[b]	**2**[b]
C-1	126.5	127.9	128.3
C-14a	147.4		149.2
C-2	134.8	135.9	136.0
C-3	126.4	127.3	127.7
C-4	127.2	128.0	128.0
C-4a	120.8		122.3
C-5	162.1		163.6
C-7	74.9	75.1	80.5
C-8	27.2	30.8	64.9
C-8a	114.4		117.8
C-8b	125.5		127.3
C-9	120.1	120.8	120.6
C-10	120.7	121.1	121.7
C-11	125.7	126.2	126.1
C-12	112.1	113.3	113.4
C-12a	138.5		140.3
C-13a	126.2		117.7
C-13b	143.2		144.9

[a] Measured in $CDCl_3$. [b] Measured in CD_3OD.

(sh), 275, 288, 332, 345, and 363 nm) and implied the presence of a chromophore very similar to those present in compounds **1** and **3**.[5] The IR spectrum (ν_{max}, $CHCl_3$) showed bands at 3300, 1662, 1610, and 1600 cm^{-1}. HREIMS data established the molecular formula $C_{18}H_{13}N_3O_3$ for **2**. EIMS fragment ions were observed at *m/z* 319 (M^+), 301 $[M - H_2O]^+$, 289, 288, and 285 [M − 34]. The loss of 34 from M^+ (319) corresponds to the molecular weight of 7,8-dehydrorutaecarpine[5] and suggested that compound **2** differs from 7,8-dehydrorutaecarpine by addition of hydroxy groups at C-7 and C-8. The ^1H NMR, COSY, HOHAHA, and HMQC spectra showed the presence of the same aromatic spin systems as in compound **1** (Tables 1 and 2). Two methine protons were observed at δ 6.70 (1H, d, $J = 2$ Hz, H-7) and 5.24 (1H, d, $J = 2$ Hz, H-8), one of which replaced the geminal proton at C-8 in compound **1**. The ^{13}C NMR signals for **2** were close to those of **1** except that **2** had two OH-substituted methine carbons at C-8 (δ 64.9) and C-7 (δ 80.5). All the expected connectivities were revealed between H-7 (δ 6.70) and C-14a (δ 144.9) and between H-8 (δ 5.24), C-9a (δ 127.3), and C-13a (δ 117.7) on the basis of long-range $^{13}C-^1H$ correlations (HMBC experiment). The orientations of the H-7 and H-8 protons were studied by NOE experiments. Due to the planarity of the indole ring, NOE correlations (7%) between the signals at δ 7.76 (H-9) and 5.24 (H-8) inferred that the orientation of this proton should be equatorial, and further correlations (17%) between the signals at δ 5.24 (H-8) and 6.70 (H-7) were observed. The coupling constant between H-7 and H-8 in **2** was relatively small (2 Hz). On the basis of the coupling constant between H-7 and H-8 in compound **1** ($J = 1.5$ Hz) and also comparison of that published for indolopyridoquinazoline-type alkaloids,[10,11] H-8 is presumed to be oriented equatorially. Consequently, it is inferred that the orientation of the protons at C-7 and C-8 should be trans diequatorial. On the basis of the above data, compound **2** was identified as the structure 7,8-dihydroxyrutaecarpine. This is the first report of **2** from a natural source.

Callus tissues of *P. amurense* are now known to produce four indolopyridoquinazoline-type alkaloids, together with furoquinoline-, canthinone-, and isoquinoline-type alkaloids, which are the main alkaloid components in the original plants.[5] The indolopyridoquinazoline-type alkaloids occur in the genera *Evodie, Hortia, Zanthoxylum*, and *Euxylophora*, all members of the Rutaceae, and are restricted to only a few species.[12,13]

However, only the isoquinoline- and canthinone-type alkaloids have been reported from the original plant, *P. amurense*.[6] In contrast, we have been unable to detect furoquinoline-type alkaloids in several kinds of original plant parts (root, stem, and fruits). Therefore, there are interesting differences in the biosynthesis of the alkaloids between the callus tissue and its original plant.

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR: 500 and 125 MHz (JEOL GSX-500), respectively, room temperature, $CDCl_3$ and CD_3OD. Chemical shifts are given in δ (ppm) with residual solvent signals (δ 7.26 and 77.0, respectively) as internal standards. Multiplicities for the ^{13}C NMR spectra were determined by DEPT experiments at 90 and 135°, and NMR assignments were determined by HOHAHA, HMQC, and HMBC experiments (Varian Unity-400 spectrometer). MS were recorded with a direct inlet probe at 70 eV (JEOL JMS−SX102A), and HRMS measured on a JEOL JMS−SX102A mass spectrometer.

Plant Material. Stems of *P. amurense* Rupr. (Rutaceae) were collected in April 1992 at the Medicinal Plant Garden of Science University of Tokyo. The plant material was identified by Dr. T. Nakamura, Faculty of Pharmaceutical Sciences, Science University of Tokyo, and a voucher specimen (no. ph-92-04) was deposited at the herbarium of our Institute.

Callus Cultures. Callus tissues from stems of *P. amurense* were established in April 1992. Murashige and Skoog[7] medium (minus glycine) (M&S) containing 2,4-D (1−3 mg/L) and kinetin (KIN) (0.1 mg /L) as plant growth regulators were used for the induction of callus tissues. The callus tissues were subcultured every 5−6 weeks onto fresh M&S medium containing 2,4-D (1 mg/L) and KIN (0.1 mg /L) at 25 ± 1 °C in the dark.

Extraction and Isolation. The *P. amurense* callus tissues subcultured at 5 week intervals on static MS medium containing 2,4-D (1 mg/L) with KIN (0.1 mg/L) as plant growth regulators during 7−8 months and callus tissues were harvested at 5-week intervals and were stored in MeOH for alkaloid investigation. The stored callus tissues (fresh weight 4.5 kg, dry weight 130 g) were extracted with cold MeOH and EtOAc in a Waring blender. The extracts were concentrated under reduced pressure, and the residue was partitioned between $CHCl_3$ (2 L) and H_2O (0.3 L) to obtain the organic-soluble fraction. The $CHCl_3$ solution was evaporated to dryness, and the extracts were chromatographed on a column of silica gel (Merck 9385) by gradient elution using $CHCl_3$ with increasing proportions of MeOH. The crude alkaloid mixtures so obtained were purified further by column chromatography on a silica gel column (Merck 9385) eluted with hexanes−EtOAc to give alkaloids **1** and **2**.

7-Hydroxyrutaecarpine (1): pale yellow powder (6.1 mg); $[\alpha]^{25}_D$ +73° (*c* 0.0012, MeOH); UV (EtOH) λ_{max} (log ϵ) 215 (4.55), 225 (4.54), 265 (sh) (4.04), 275 (3.96), 288 (3.97), 330 (4.56), 345 (4.73), 363 (4.46) nm; IR ($CHCl_3$) ν_{max} 3345, 1690, 1600, 1475, 1395 cm^{-1}; EIMS

m/*z* 303 $[M]^+$ (30), 285 $[M - 18]^+$ (53), 275 (100), 155 (10); HREIMS *m*/*z* 303.1003 (calcd for $C_{18}H_{13}O_2N_3$, 303.1008).

7,8-Dihydroxyrutaecarpine (2): colorless needles (EtOAc−EtOH) (8.2 mg); mp 247−250 °C; $[\alpha]^{25}_D$ −38° (*c* 0.0016, MeOH); UV (EtOH) λ_{max} (log ϵ) 215 (4.77), 240 (sh) (4.63), 275 (4.19), 288 (4.16), 332 (4.64), 345 (4.70), 362 (4.60) nm; IR ($CHCl_3$) ν_{max} 3300, 1662, 1610, 1600 (C═C), 1475, 1335 cm^{-1}; EIMS *m*/*z* 319 $[M]^+$ (54), 301 $[M - H_2O]^+$ (48), 290 (47), 289 (55), 288 (100), 285 (15), 267 (10), 261 (52), 170 (7), 169 (22), 146 (21), 119 (26,); HREIMS *m*/*z* 319.0958 (calcd for $C_{18}H_{13}O_3N_3$, 319.0962).

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