7-HYDROXYRUTAECARPINE FROM *TETRADIUM GLABRIFOLIUM* AND *TETRADIUM RUTICARPUM*

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Abstract -- A new quinazolinocarboline alkaloid derivative, 7-hydroxyrutaecarpine (**1**), was isolated from the heartwood of *Tetradium glabrifolium* and the fruit of *Tetradium ruticarpum.*

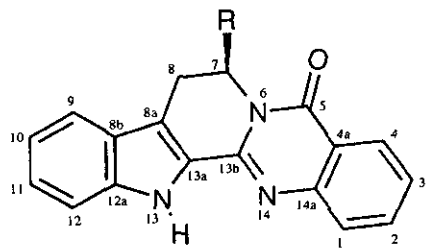
INTRODUCTION

Tetradium glabrifolium (*Evodia meliaefolia*) (Rutaceae), a large shrub or small to medium sized tree, is especially widespread in southern part of Taiwan.[1] It is used to treat chronic ulcer of lower extremity and stomach ache.[2] For taxonomic reason, forty-six compounds including six benzo[c]phenanthridines, four furoquinolines, two quinazolinocarbolines, two 2-quinolones, one sterol, one steroidal glucoside, one sesquiterpenoid, one triterpenoid, one lignin, one coumarin, fifteen benzenoids, six tetranortriterpenoids and five amides have been isolated from the heartwood of *T. glabrifolium*.[3] On the other hand, four quinazolinocaboline alkaloids, one limonoid and three flavonoids have been obtained from the fruit of *T. ruticarpum*.[4] Further examination of the methanolic extracts from the heartwood of *T. glabrifolium* and the ethanolic extract from the fruit of *T. ruticarpum*. a new quinazolinocarboline alkaloid, 7-

hydroxyrutaecarpine (**1**), was isolated and identified by spectral analyses at the same time. Here we report the structural elucidation of **1**.

RESULTS AND DISCUSSION

7-Hydroxyrutaecarpine (**1**), an optically active compound, $[\alpha]_D$ + 187.8° (c = 0.0017, MeOH), was isolated as colorless needles with mp 228-230 °C. The high resolution mass spectrum established a molecular formula as $C_{18}H_{13}N_3O_2$ (*m/z* found: 303.1007, calcd: 303.1008). The uv (MeOH) absorptions at 223.8 (4.25), 226.4 (4.26), 270.6 (3.61), 275.4 (3.63), 332.2 (4.24), 346.2 (4.28), 364.0 (4.15) nm as well as amidic and aromatic bands at 1647, 1595, 1553 cm^{-1} in ir (KBr) spectrum together with an NH signal at δ 11.78 (exchangeable with D_2O) in ^1H nmr (DMSO-d_6) spectrum were very close to that of rutaecarpine (**2**).[5] These data indicated that compound (**1**) should be a quinazolinocarboline alkaloid derivative.[6]



1: R = OH
2: R =H
3: R = COOH

In the aromatic region of ^1H nmr spectrum, two pairs of four-mutually-coupled protons at δ 7.08 (dd, *J* = 7.7, 7.7 Hz, H-10), 7.26 (dd, *J* = 7.7, 7.7 Hz, H-11), 7.50 (d, *J* = 7.7 Hz, H-12), 7.65 (d, *J* = 7.7 Hz, H-9) and 7.48 (dd, *J* = 8.1, 8.1 Hz, H-3), 7.68 (d, *J* = 8.1 Hz, H-1), 7.82 (ddd, *J* = 8.1, 8.1, 1.2 Hz, H-2), 8.19 (dd, *J* = 8.1, 1.2 Hz, H-4) revealed that ring A and E were unsubstituted. Another D_2O exchangeable signal at δ 6.70 (d, *J* = 5.1 Hz) was assigned as hydroxyl group attached at C-7 which could be proved by the characteristic fragment ion at *m/z* 285 (M^+ - H_2O) in ms spectrum. In addition, a ABX pattern at δ 3.22 (dd, *J* = 17.6, 5.1 Hz, H-8_{ax}), 3.39 (dd, *J* = 17.6, 1.2 Hz, H-8_{eq}) and 6.77 (ddd, *J* =

5.1, 5.1, 1.2 Hz, H-7_{eq}) showed the presence of geminal protons at C-8 and a methine proton at C-7 very deshielded by two heteroatoms, N and O. These assignments were confirmed by ^{1}H-^{1}H COSY and HMQC spectra.

The stereochemistry for H-7 was suggested from the small coupling constant values of this proton with H-8_{ax} and H-8_{eq} which accounted for the equatorial orientation of H-7 and an axially oriented hydroxyl substituent. This was analogus as that of tryptophan-derived indolopyridoquinazoline alkaloid (**3**).[7]

All the expected connectivities, especially the orientation of two H-8 protons, were further studied by NOESY (Figure 1) and HMBC (Figure 2) experiments. Due to the planarity of the indole ring moiety, the existence of NOE correlation between signals at δ 7.65 (H-9) and 3.39 (H-8) inferred the orientation of this proton should be equatorial. Consequently, H-8 at δ 3.22 oriented axially. On the basis of the above data, the structure of 7-hydroxyrutaecarpine was determined as **1**.

Except benzo[*c*]phenanthridines, the quinazolinocarboline alkaloids can also be acted as a mark for chemotaxonomy and support Hartley's decision to reassign taxa from *Evodia* into three genera: *Tetradium*, *Evodia s. s.* and *Melicope*.[8]

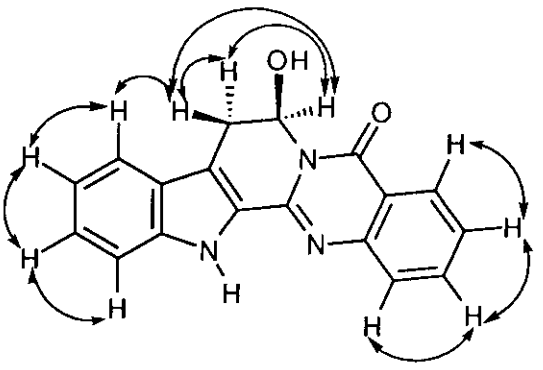


Figure 1 NOESY correlations for 7-hydroxyrutaecarpine (**1**)

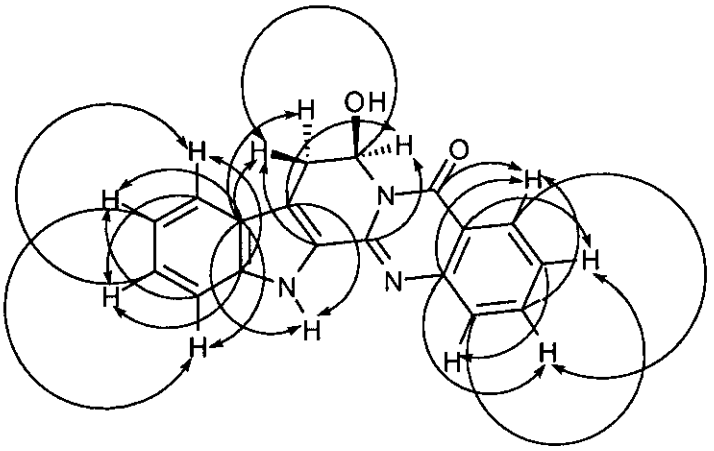


Figure 2. ^{13}C-^{1}H long range correlations from the HMBC experiment of 7-hydroxyrutaecarpine (**1**)

EXPERIMENTAL

Melting point (Yanaco apparatus) is uncorrected. Ir spectrum was recorded on a Jasco IR Report-100 spectrophotometer. Uv spectrum was obtained on a Hitachi UV-3210 spectrophotometer. ^{1}H nmr and ^{13}C nmr spectra as well as 2D spectra were determined on Bruker AC-200, AMX-400 and Varian Unity Plus-400 spectrometers. Chemical shifts are shown in δ values (ppm) with tetramethylsilane as internal standard. Ms spectrum was measured on VG-70-230S spectrometer.

Plant Material

Tetradium glabrifolium (Champ. *ex* Benth) T. Hartley (Rutaceae) was collected from San Dei Men, Pingtung Hsien, Taiwan and verified by Professor C. S. Kuoh (National Cheng Kung University, R.O.C.), whereas the fruit of *Tetradium ruticarpum.* was purchased from market and verified by Professor I. T. Wang (Cheng Du College of Medicine, China).

Extraction and Separation

The dried heartwood (15 kg) of *T. glabrifolium* was extracted with hot methanol (140 l) for 10 h. The methanolic extract was concentrated to give a brown syrup (402 g) which was partitioned between H_2O (2

l) and $CHCl_3$ (8 l) and then between H_2O (2 l) and *n*-BuOH (4 l). The $CHCl_3$ extract (98 g) was chromatographed on silica gel using $CHCl_3$-$(CH_3)_2CO$ (9:1) as eluent to afford nine fractions. Fractions 3 (2.3 g) and 4 (1.7 g) were combined and rechromatographed on silica gel to give 7-hydroxyrutaecarpine (**1**) (1.7 mg). The separation of fractions 1, 2 and 5-9 was reported in the previous paper.[3]

In addition, the ethanolic extract (30 l x 4) from the fruit of *T. ruticarpum* (9 kg) was concentrated and chromatographed on Amberlite XAD-2 eluting with H_2O, H_2O-MeOH (1:1), MeOH, MeOH-$CHCl_3$ (1:1) and $CHCl_3$, successively. The MeOH-$CHCl_3$ fraction was concentrated to 500 ml. After filtration, the precipitate (10.5 g) was recrystallized from $CHCl_3$/MeOH to give rutaecarpine (**2**). The filtrate (2.6 g) was repeated rechromatographed on silica gel and eluted with $CHCl_3$ to give the same compound (**1**), 7-hydroxyrutaecarpine (23 mg).

*7-Hydroxyrutaecarpine (**1**)*

Ir υ_{max} (KBr) cm^{-1} 3300, 1647, 1595, 1553; ms *m/z* (rel. int.) 303 (M^+, 29), 286 (33), 285 (32), 275 (100), 274 (78); ^1H nmr (DMSO-d_6, 300 MHz) δ 3.22 (1H, dd, *J* = 17.6, 5.1 Hz, H-8_{ax}), 3.39 (1H, dd, *J* = 17.6, 1.2 Hz, H-8_{eq}), 6.70 (1H, d, *J* = 5.1 Hz, 7-OH), 6.77 (1H, ddd, *J* = 5.1, 5.1, 1.2 Hz, H-7_{eq}), 7.08 (1H, dd, *J* = 7.7, 7.7 Hz, H-10), 7.26 (1H, dd, *J* = 7.7, 7.7 Hz, H-11), 7.48 (1H, dd, *J* = 8.1, 8.1 Hz, H-3), 7.50 (1H, d, *J* = 7.7 Hz, H-12), 7.65 (1H, d, *J* = 7.7 Hz, H-9), 7.68 (1H, d, J = 8.1 Hz, H-1), 7.82 (1H, ddd, *J* = 8.1, 8.1, 1.2 Hz, H-2), 8.19 (1H, dd, *J* = 8.1, 1.2 Hz, H-4), 11.78 (1H, br s, NH); ^1H nmr ($CDCl_3$, 400 MHz) δ 3.44 (1H, dd, *J* = 17.6, 5.6 Hz, H-8_{ax}), 3.59 (1H, dd, *J* = 17.6, 1.6 Hz, H-8_{eq}), 4.23 (1H, br s, 7-OH), 6.87 (1H, dd, *J* = 5.6, 1.6 Hz, H-7_{eq}), 7.18 (1H, dd, *J* = 7.8, 7.8 Hz, H-10), 7.34 (1H, dd, *J* = 7.8, 7.8 Hz, H-11), 7.41 (1H, dd, *J* = 8.0, 8.0 Hz, H-3), 7.45 (1H, d, *J* = 7.8 Hz, H-12), 7.62 (1H, d, *J* = 8.0 Hz, H-1), 7.64 (1H, d, *J* = 7.8 Hz, H-9), 7.73 (1H, dd, *J* = 8.0, 8.0 Hz, H-2), 8.26 (1H, d, *J* = 8.0 Hz, H-4), 9.20 (1H, br s, NH), ; ^{13}C nmr (DMSO-d_6, 75 MHz) δ 160.2 (s, C-5), 147.4 (s, C-14a), 144.0 (s, C-13b), 138.7 (s, C-12a), 134.6 (d, C-2), 126.7 (d, C-4),, 126.4 (d, C-1), 125.9 (d, s, C-3, C-13a), 125.8 (s, C-8b), 124.8 (d, C-11), 120.8 (s, C-4a), 119.7 (d, C-9), 119.5 (d, C-10), 114.7 (s, C-8a), 112.4 (d, C-12), 73.2 (d, C-7), 27.3 (t, C-8).

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