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Determination of the Absolute Configuration of Rohitukine

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Abstract: The absolute configuration of rohitukine, isolated from the stem-bark of *Dysoxylum binectariferum*, was determined to be 5,7-dihydroxy-2-methyl-8-[4-(3S, 4R-3-hydroxy-1-methyl) -piperidinyl]-4H-1-benzopyran-4-one by X-ray crystallographic analysis on the crystal of 4-bromobenzoyl derivatives of rohitukine. At the same time, the modified Mosher′ method was proved to be unsuitable for determining the absolute configuration of C-3′ position in rohitukine.

Keywords: *Dysoxylum binectariferum*, absolute configuration, rohitukine.

Rohitukine 5, 7-dihydroxy-2-methyl-8- [4-(3-hydroxy-1-methyl)-piperidinyl]- 4H-1-benzopyran-4-one, had been reported to have the anti-inflammatory activity in the carrageenin-induced rat paw oedema assay (ED_{50}=9 mg/kg, p.o.) and inhibit the reverse passive Arthus reaction in rats (50.8±5.9%, inhibition at 2.5 mg/kg, p.o.) [1]. Its structure and relative configuration were determined by X-ray crystallographic analysis[2], but its absolute configurations of the chiral center at C-3′ and C-4′ position were still unknown. This paper reports the determination of the absolute configuration of the chiral centers in rohitukine.

To isolate rohitukine, the stem-bark of *Dysoxylum binectariferum* (Roxb.) Hook. f. ex Bedd (which was collected from Ledong County, Hainan Province of China and identified by professor H. B. Chen in School of Pharmaceutical Sciences, Peking University), was extracted with methanol. The MeOH extract was concentrated and the residue was suspended in water, and extracted with EtOAc and *n*-BuOH successively. *n*-BuOH fraction (2 g) was partitioned between 1 mol/L hydrochloric acid (100 mL) with ethyl acetate (100 mL), the acid water-layer was basified to pH 10 by 29% ammonia solution, and extracted with *n*-BuOH, to obtain the alkaloid portion (196.5 mg). *n*-BuOH extract was separated by low-pressure column chromatography [column: YMC Gel-ODS (120~150 μm); mobile phase: 30% acetonitrile-70% phosphate buffer (pH 3.5)], detected by UV (254 nm) and Dragendorff reagent. The same fractions were collected and desalted with HP-20. One compound (55.90 mg) was isolated. Based on FABMS (M.W. 305), ^{1}H NMR (**Table 1**), ^{1}H-^{1}H COSY, NOESY (**Figure 1**) spectra and literature[2], it′s structure and relative configuration were determined to be (3′S,

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4′R) or (3′R, 4′S) -rohitukine.

In order to determine the absolute configuration of chiral center at C-3′ and C-4′ in rohitukine, we tried the modified Mosher ester methodology[3] first, used MTPA (2-methoxy-2-trifluoromethyl-2-phenyl acetyl chloride) as chiral anisotropic reagent to determine the absolute configuration of secondary alcohol. (R) and (S) -rohitukine-MTPA ester were synthesized by the usual method. Based on on-line UV detection, (R) or (S) -rohitukine-MTPA ester was separated by HPLC [column: J′Sphere ODS M-80 (20×250 mm); mobile phase: 20% acetonitrile/80% phosphate buffer (pH 3.5) → 80% acetonitrile/20% phosphate buffer (pH 3.5), 50 min, liner gradient]. Based on MS and ^{1}H NMR spectra, the structure of rohitukine-MTPA ester was identified to be 3′-rohitukine-MTPA ester. The $\Delta\delta_H$ (S-R) data of (S)- and (R)- rohitukine-MTPA ester showed that the difference of δ_H (S-R) was in irregular orders between the two sides of the MTPA planes (**Table 1** and **Figure 2**). So, the modified Mosher′ method was proved to be unsuitable for determining the absolute configuration of C-3′ in rohitukine.

Table 1 ^{1}H NMR data of rohitukine, (S) or (R)- rohitukine-MTPA ester, and value of Δ (S-R)

H	rohitukine	(S) -MTPA	(R) -MTPA	Δ (S-R)
a	6.136 (s)	6.08	6.26	− 0.18
b	5.986 (s)	5.79	5.95	− 0.16
c	4.211 (br)	5.23	5.27	− 0.04
d	3.627 (dm, 9.53)	3.51	3.73	− 0.22
e	3.440 (dm, 12.2)	3.26	3.21	+ 0.04
f	3.517 (dm, 8.3)	3.02	2.98	+ 0.04
g	3.156 (m)	2.90	2.73	+ 0.17
h	3.315 (dm, 11.23)	2.43	2.48	− 0.05
i	2.860 (s)	2.30	2.32	− 0.02
j	2.374 (s)	2.22	2.25	− 0.03
k	3.135 (m)	2.13	2.23	− 0.10
l	1.714 (dm, 10.25)	1.71	1.83	− 0.12

Figure 1 ^{1}H-^{1}H COSY and NOESY correlation of rohitukine

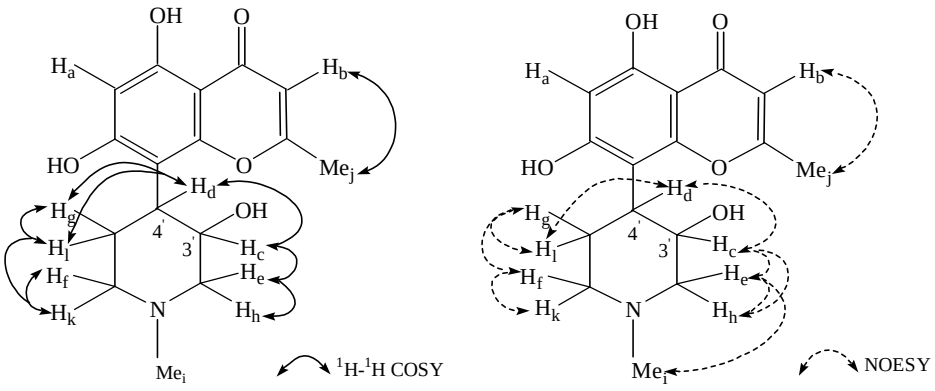
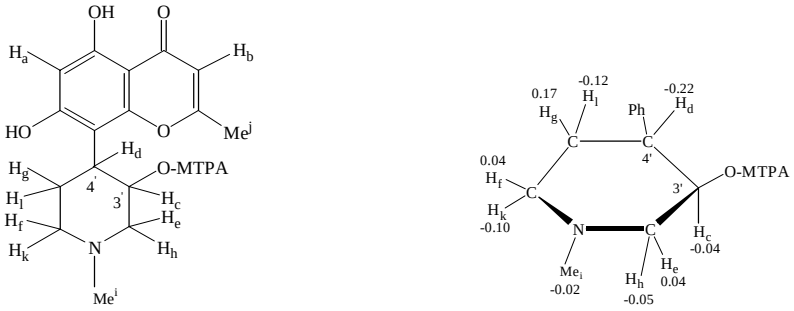
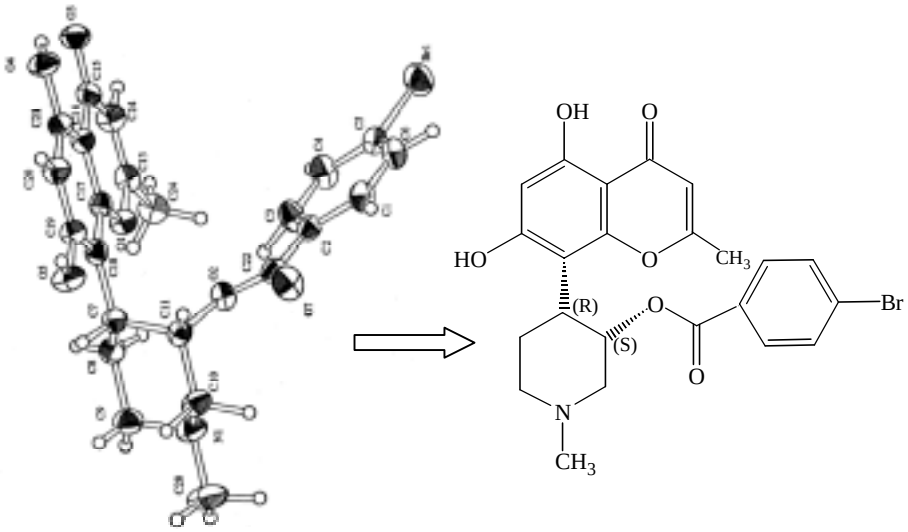


Figure 2 Structure of rohitukine-MTPA ester and difference value of ^{1}HNMR data of (S) and (R)- rohitukine-MTPA ester



Therefore, the method of X-ray crystallographic analysis on the crystal of 4-bromobenzoyl derivatives of rohitukine was tried sequentially. The derivative was synthesized by the usual way using rohitukine and 4-bromobenzoyl chloride, and the crystal was obtained from ethanol solution. By X-ray crystallographic analysis, the absolute configuration of chiral center at C-3′ and C-4′ in rohitukine was determined as C-3′S and C-4′R (**Figure 3**).

Figure 3 X-ray crystallogram of 4-bromobenzoyl derivative of rohitukine



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