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RESCINNAMINOL – A NEW ALKALOID FROM *RAUWOLFIA SERPENTINA* BENTH

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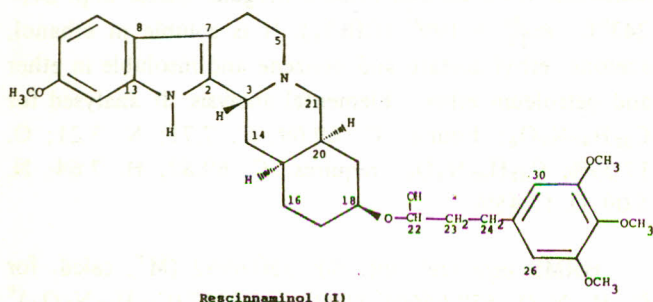
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A new yohambanoide, rescinnaminol, has been isolated from the roots of *Rauwolfia serpentina* Benth of Thai origin and its structure has been elucidated through spectroscopic studies.

Key words: *Rauwolfia serpentina*; Rescinnaminol, Yohambanoide

INTRODUCTION

The present work was undertaken in the alkaloidal constituents of the roots of *Rauwolfia serpentina* Benth in the light of earlier observations that alkaloidal constituents show significant regional variation [1-4]. As a result, a new base, rescinnaminol, has been isolated by employing a mild isolation procedure as described in the experimental section.



RESULTS AND DISCUSSION

The ethyl acetate soluble bases were fractionated into 1% and 2% acetic acid soluble fractions. The bases obtained from the 1% acetic acid soluble fraction according to the procedure described in the experimental section, were chromatographed on thin layer plates of silica gel (GF-254, 0.2mm) when a yellowish crystalline base was obtained, which formed irregular plates on recrystallization from alcohol and melted at 241-243°C, $[\alpha]_D^{20} + 199^\circ$ (CHCl_3).

The UV spectrum exhibited absorption at λ_{max} 210, 222 and 285 nm indicating the presence of an indolic chromophore [5]. The IR spectrum showed the presence of

hydroxyl group at 3410 (O-H stretching), 1380 cm^{-1} (O-H bending) and aromatic vibrations at 3100 and 1465 cm^{-1} , whereas no band was observed in the range of 1650-1800 cm^{-1} showing the absence of any carbonyl group. Furthermore, the absence of Wenkert-Bohlman bands (2700-2800 cm^{-1}) suggested the presence of a *cis*-quinolizidine system [6, 7]. The electron impact (EI) mass spectrum showed the molecular ion peak at m/e 550 which was confirmed by high resolution mass at m/e 550.6562 corresponding to the molecular formula $\text{C}_{32}\text{H}_{42}\text{N}_2\text{O}_6$. A peak at m/e 549 (M^+-1), due to the expulsion of H-3 indicated that the alkaloid belongs to the yohimbine family [8]. The peak at m/e 382.2256 ($\text{C}_{23}\text{H}_{30}\text{N}_2\text{O}_3$)⁺ resulted from the loss of trimethoxy phenyl group which on further loss of a water molecule gave a peak at m/e 364.2150. ($\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_2$)⁺. The peak at m/e 267.1498 ($\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}$)⁺ possibly results from the elimination of C-16 to C-18 along with C-18 substituent and the peaks at m/e 255.1496 ($\text{C}_{16}\text{H}_{19}\text{N}_2\text{O}$)⁺ and 251.1184 ($\text{C}_{16}\text{H}_{15}\text{N}_2\text{O}$)⁺, arise from the cleavage of D/E ring junction and further loss of two protons respectively. [9]

The peak at m/e 214.1106 ($\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}$)⁺, 200.0949 ($\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}$)⁺, 186.0918 ($\text{C}_{12}\text{H}_{12}\text{NO}$)⁺ and 174.0918 ($\text{C}_{11}\text{H}_{12}\text{NO}$)⁺ supported the presence of β -carboline system in the molecule [10].

The ¹H-NMR spectrum afforded two doublets at δ 7.31 ($J_{9, 10} = 8.5$ Hz) and 6.83 ($J = 2.1$ Hz), a double doublet at δ 6.67 ($J_{9, 10} = 8.5$ Hz, $J_{10, 12} = 2.1$ Hz) and a singlet at δ 6.42 attributable to aromatic protons H-9, H-12, H-10 and H-26 and H-30, respectively. The chemical shifts of H-9, H-10 and H-12 and the absence of H-11 signal showed that the methoxy group which was indicated by the mass spectrum to be located at one of the β -carboline carbons, is present at C-11. A one-proton triplet ($J = 7.1$ Hz) at δ 5.25 assigned to H-22 indicated the presence of two oxygen functions at C-22 while H-18 resonated at δ 4.75 as a

doublet of quartet ($J_{17\alpha, 18} = 2.2$ Hz, $J_{17\beta, 18} = 9.4$ Hz, $J_{18, 19\alpha} = 4.9$ Hz, $J_{18, 19\beta} = 11.6$ Hz). H-3 appeared at δ 4.45 as a broad singlet and an upfield signal at δ 1.82 has been assigned to H-20. The down field chemical shift of H-3 indicated its β -disposition [11] and the values of coupling constants provided evidence for the α -orientation of H-18 and H-20. H-23 and H-24 appeared at δ 2.13 and 2.40 as a multiplet and a triplet ($J = 7.0$ Hz) respectively, showing the absence of unsaturation between C-23 and C-24 as noted in the case of rescinnamidine. Three sharp singlets at δ 3.85, 3.83 and 3.45 integrated for twelve protons of four methoxy groups. On the basis of these spectral data, structure has been assigned to rescinnaminol, which was further supported by the ^{13}C -NMR spectrum (Table 1). The signals at δ 51.77 (C-3), 33.52 (C-15) and 34.07 (C-20) in the ^{13}C -NMR spectrum substantiated the epiallo configuration of rescinnaminol [12, 13].

Table. ^{13}C -NMR chemical shift assignments.

Carbons	Chemical shifts	Carbons	Chemical shifts
2	a	18	77.77
3	51.77	19	29.58
5	51.23	20	34.07
6	16.86	21	49.12
7	a	22	105.59
8	a	23	37.02
9	118.61	24	26.76
10	112.36	25	a
11	a	26,30	109.18
12	95.37	27,29	a
13	a	28	a
14	24.32	11-OCH ₃	53.78
15	33.52	27,29-OCH ₃	55.92
16	27.57	28-OCH ₃	60.74
17	28.96		

All values are in ppm relative to TMS = 0

a. Signal not detected.

EXPERIMENTAL

Melting point was recorded in glass capillary tube and was uncorrected. IR (chloroform) and UV (methanol) spectra were measured on JASCO IRA-1 spectrometer and Pye-Unicam SP-800 Spectrometer respectively. Mass spectra were recorded on Finnigan MAT 112 and 312 double focusing mass spectrometers connected to PDP 11/34 computer system. ^1H and ^{13}C -NMR (Broad Band and

Gated Spin Echo) spectra recorded in CDCl_3 with TMS as internal reference on a Brüker Aspect 3000 instrument operating at 300 MHz. The purity of the sample was checked on TLC (silica gel S.I.F. - 254) precoated aluminium cards.

Isolation. Freshly collected undried roots of *R. serpentina* Benth (6 kg) were extracted repeatedly with ethanol after prior overnight dip in water. The roots were then air dried and reduced to 50 mesh. The powdered material was repeatedly percolated with ethanol in the course of over two weeks. The combined alcoholic extracts were freed of the solvent under reduced pressure below 40° . The residue was taken up in water and exhaustively shaken out with ethyl acetate finally with the addition of 5% acetic acid. The ethyl acetate phase was washed with water, dried (Na_2SO_4) charcoaled and freed of the solvent affording a light yellow semi-solid residue which was defatted with petroleum ether and divided into ether benzene soluble and ethyl acetate soluble fraction. The latter was shaken out with 1% acetic acid, which ammoniated to pH-8 and liberated bases were extracted in ethyl acetate, which on thin layer chromatography [silica gel, 0.2 mm chloroform-ethyl acetate-ethanol (8:1.7:0.3)] yielded a new base rescinnaminol as yellow crystalline solid which on recrystallization from alcohol formed irregular plates m.p. $241-243^\circ\text{C}$, $[\alpha]_D^{20} + 199^\circ$ (CHCl_3). It is soluble in ethanol, acetone, ethyl acetate and benzene and insoluble in ether and petroleum ether. Elemental analysis: It analysed for $\text{C}_{32}\text{H}_{42}\text{N}_2\text{O}_6$; Found: C, 69.69; H, 7.71; N, 5.21; O, 17.39%; $\text{C}_{32}\text{H}_{42}\text{N}_2\text{O}_6$; requires: C, 69.82; H, 7.64; N, 5.09; O, 17.45%.

HRMS m/e (rel. int. %): 550.6562 (M^+ , calcd. for $\text{C}_{32}\text{H}_{42}\text{N}_2\text{O}_6$ 550.6560) $^+$ (100), 535.6217 ($\text{C}_{31}\text{H}_{39}\text{N}_2\text{O}_6$) $^+$ (5), 519.6262 ($\text{C}_{31}\text{H}_{39}\text{N}_2\text{O}_5$) $^+$ (5), 382.2256 ($\text{C}_{23}\text{H}_{30}\text{N}_2\text{O}_3$) $^+$ (18), 364.2150 ($\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_2$) $^+$ (10), 278.1782 ($\text{C}_{19}\text{H}_{22}\text{N}_2$) $^+$ (8), 267.1498 ($\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}$) $^+$ (20), 255.1496 ($\text{C}_{16}\text{H}_{19}\text{N}_2\text{O}$) $^+$ (18), 251.1184 ($\text{C}_{16}\text{H}_{15}\text{N}_2\text{O}$) $^+$ (12), 214.1106 ($\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}$) $^+$ (20), 212.0949 ($\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}$) $^+$ (14), 200.0949 ($\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}$) $^+$ (16), 186.0918 ($\text{C}_{12}\text{H}_{12}\text{NO}$) $^+$ (10) and 174.0918 ($\text{C}_{11}\text{H}_{12}\text{NO}$) $^+$ (10).

IR ν_{max} (cm^{-1}): 3410 (O-H stretching), 1380 (O-H bending) and 3100, 1465 (aromatic vibrations). UV λ_{max} (nm): 210, 222 and 285. ^1H -NMR (CDCl_3): δ 7.71 (1H, s, NH), 7.31 (1H, d, $J_{9, 10} = 8.5$ Hz, H-9), 6.83 (1H, d, $J_{10, 12} = 2.1$ Hz, H-12), 6.67 (1H, dd, $J_{9, 10} = 8.5$ Hz, $J_{10, 12} = 2.1$ Hz, H-10), 6.42 (2H, s, H-26 & 30), 5.25 (1H, t, $J_{22, 23} = 7.1$ Hz, H-22), 4.75 (1H, dq, $J_{17\alpha, 18} = 2.2$ Hz, $J_{17\beta, 18} =$

9.4 Hz, J_{18, 19α} = 4.9 Hz, J_{18, 19β} = 11.6 Hz, H-18), 4.45 (1H, brs, H-3β), 3.85 (6H, s, OCH₃), 3.83 (3H, s, OCH₃), 3.45 (3H, s, OCH₃), 3.16 (2H, m, H-5), 3.06 (1H, dd, J_{20, 21β} = 4.0 Hz, J_{21α, 21β} = 12.0 Hz, H-21 β), 2.95 (1H, m, H-6α), 2.61 (1H, J_{20, 21α} = 4.0 Hz, J_{21α, 21β} = 12.0 Hz, H-21 α), 2.44 (1H, m, H-6 β), 2.40 (2H, t, J = 7.0 Hz, H-24), 2.35 (1H, m, H-17β), 2.25 (1H, dd, J_{15α, 16β} = 5.4 Hz, J_{16β, 17α} = 5.1 Hz, J_{16α, 16β} = 12.9 Hz, H-16 β), 2.15 (1H, m, H-19 β), 2.13 (1H, m, H-23), 2.01 (1H, dddd, J_{16α, 17α} = 3.9 Hz, J_{16β, 17} = 5.1 Hz, J_{17α, 18} = 2.2 Hz, J_{17α, 17β} = 13.0 Hz, H-17 α), 1.91 (1H, dt, J_{15, 16α} = 3.6 Hz, J_{15, 16β} = 5.4 Hz, J_{14, 15} = 12 Hz, J_{15, 20} = 4.5 Hz, H-15 α), and 1.82 (1H, m, H-20 α).

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