STRUCTURE AND SYNTHESIS OF A NEW CHIRAL β-CARBOLINE FOUND IN CULTURED CELL CLUMPS OF *RAUWOLFIA SERPENTINA* AND *RHAZYA STRICTA*

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The structure of a new chiral β-carboline derivative, Compound D, which was found during the chemical investigation of metabolites formed by cultured hybrid cells of two Apocynaceae plants, *Rauwolfia serpentina* Benth. and *Rhazya stricta* Decaisne, was rigorously confirmed by chemical synthesis starting from tryptamine and D-glucose.

KEY WORDS hybrid cell; β-carboline; total synthesis; absolute configuration; *Rauwolfia serpentina*; *Rhazya stricta*

During our chemical studies of secondary metabolites produced by plant cell cultures,[1] we have recently carried out an investigation[2] of the cultured hybrid cells of two Apocynaceae plants, *Rauwolfia serpentina* Benth.[3] and *Rhazya stricta* Decaisne.[3] From hybrid cell suspensions, two known monoterpenoid indole alkaloids, three known β-carboline compounds, and one new β-carboline alkaloid (Compound D) were isolated. Except for 16(*R*)-19,20(*E*)-isositsirikine, the alkaloids in the hybrid cells have not been isolated from the parental plants, callus, or cell suspension cultures. This paper describes the structure elucidation of Compound D using a synthetic method.

The plane structure of Compound D was deduced by the spectroscopic analyses, as described in a previous paper.[2] In order to determine the relative and absolute configurations of the C-3' and C-4' positions, we planned the chemical synthesis of Compound D. D-Glucose (**2**) was chosen as the starting material, because the nutritive medium used for the cultivation of the hybrid cells is rich in L-tryptophan and sucrose, and D-glucose is formed from the latter, as has recently been demonstrated by *in vivo* NMR.[4]

D-Glucose (**2**) was converted to the α, β-unsaturated aldehyde (**3**) *via* a five-step operation according to the previously reported procedure.[5] The aldehyde (**3**) was then condensed with tryptamine (**4**) in the presence of CF_3COOH in CH_2Cl_2[6] at 0°C to yield the tetrahydro-β-carboline (**5**) in 83% yield. The C ring of compound **5** was aromatized with DDQ in THF at 0°C to give the β-carboline (**6**) in 56% yield. The UV spectrum of **6** (λ_{max}: 221, 242, 280, 299, 367 nm) indicated the presence of a 1-vinyl-β-carboline system. In the ^{1}H-NMR spectrum, 3-H and 4-

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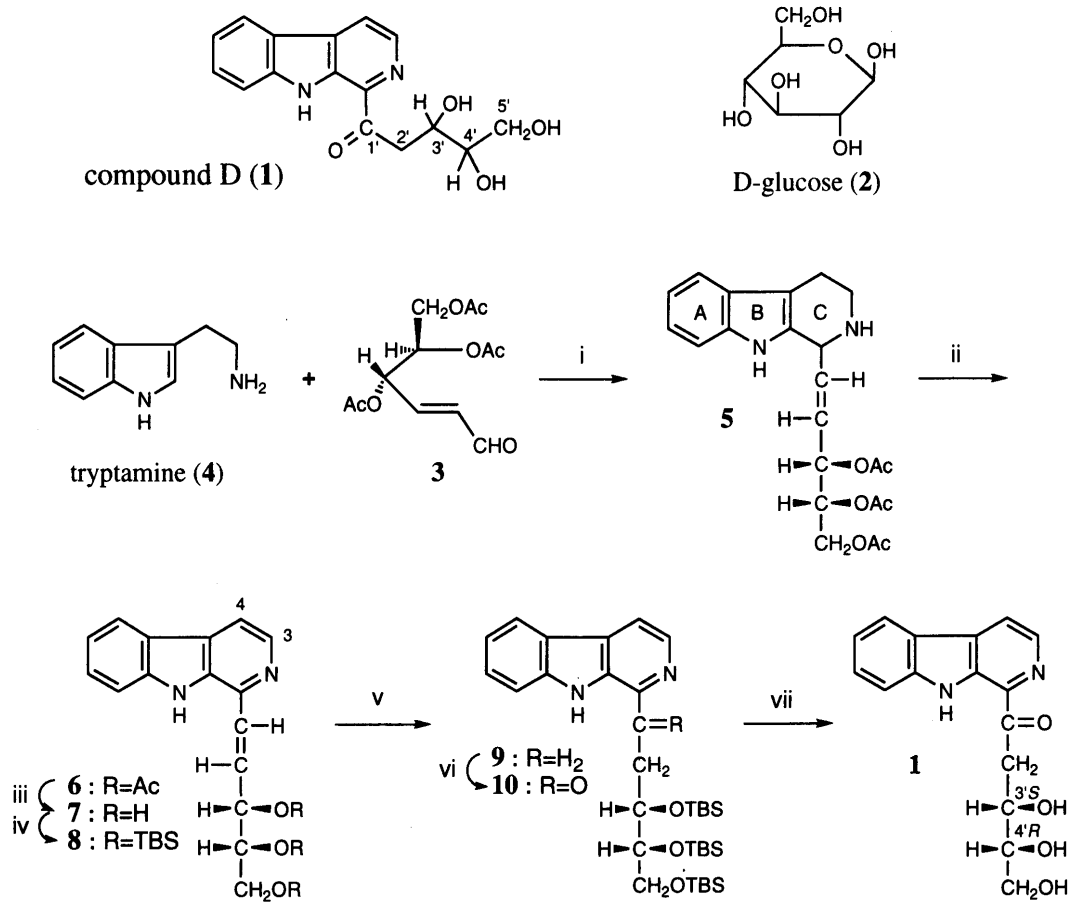
H protons in **6** were observed at δ 8.44 (d, J = 5.1 Hz) and δ 7.88 (d, J = 5.1 Hz), respectively. Next, the protecting groups of the hydroxyl functions in compound **6** were replaced with *t*-butyldimethylsilyl (TBS) groups. Thus after removal of the acetyl groups in **6** by alkaline hydrolysis (K_2CO_3, MeOH), the resulting alcohol (**7**) was treated with TBSCl, Et_3N, and DMAP in DMF to give the TBS ether (**8**) in 82% yield. Compound **8** was hydrogenated over Pd-C in EtOH to give compound **9** in 90% yield. The UV spectrum of **9** exhibited the typical absorption of the β-carboline skeleton (λ_{max}: 214, 235, 249 (sh), 283 (sh), 288, 336, 348 nm). The arylic position in **9** was oxidized with SeO_2 in 1,4-dioxane[7] at 100°C to afford the carbonyl compound (**10**) in 49% yield accompanied by the dehydrogenated compound (**8**). The UV spectrum of **10** (λ_{max}: 218, 244 (sh), 251, 262, 284, 305, 380 nm) was similar to that of Compound D. The ^{13}C-NMR spectrum of **10** showed the presence of carbonyl (C-1') and methylene (C-2') carbons at δ 203.9 and δ 41.7, respectively. Finally, the TBS ether in **10** was cleaved by treatment with tetrabutylammonium fluoride in THF to give **1** in 75% yield. The synthetic compound (**1**)[8] that has the 3'*S* and 4'*R* configuration was identical with the natural Compound D, as confirmed by the comparison of their TLC behaviors, $[\alpha]_D$, MS, and ^1H- and ^{13}C-NMR spectra.



i) CF_3COOH, CH_2Cl_2, 0°C, 1.5 h, y. 83%. ii) DDQ, THF, 0°C, 24 h, y. 56%. iii) K_2CO_3, MeOH, rt, 1 h, y. quant. iv) TBSCl, Et_3N, DMAP, DMF, 0°C, 1 h, rt, 20.5 h, y. 82%. v) H_2, 10% Pd-C, EtOH, rt, 51 h, y. 90%. vi) SeO_2, 1,4-dioxane, 100°C, 5 h, y. 49%. vii) *n*-Bu_4NF, THF, rt, 1.5 h, y. 75%.

The alkaloid **1** could also be isolated from the freshly prepared and sterilized culture medium. Therefore, it could be formed as an artificial molecule through condensation of tryptophan and a sugar unit such as glucose, followed by a series of feasible chemical reactions under rather mild conditions.

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8) $[\alpha]_D^{23}$ -25° (*c* 0.125, MeOH). UV λ_{max} (MeOH) nm: 217, 233 (sh), 242, 251, 259, 285, 308, 379. EI-MS *m/z* (%): 300 (M^+, 15), 282 (9), 251 (9), 239 (40), 221 (21), 211 (100), 182 (18), 168 (50). ^{1}H-NMR (500 MHz, CD_3OD) δ: 3.61-3.68 (m, 4H, 2'-H_2, 4'-, 5'-H), 3.81 (m, 5'-H), 4.34 (m, 1H, 3'-H), 7.31 (br-t, 1H, *J* = 7.1 Hz, 6-H), 7.59 (ddd, 1H, *J* = 8.3, 7.1, 1.2 Hz, 7-H), 7.70 (d, 1H, *J* = 8.3 Hz, 8-H), 8.22 (d, 1H, *J* = 8.1 Hz, 5-H), 8.30 (d, 1H, *J* = 4.9 Hz, 4-H), 8.46 (d, 1H, *J* = 4.8 Hz, 3-H). ^{13}C-NMR (125 MHz, CD_3OD) δ: 137.4 (C-1), 138.5 (C-3), 120.1 (C-4), 122.7 (C-5), 121.6 (C-6), 130.3 (C-7), 113.5 (C-8), 136.3 (C-10), 133.3 (C-11), 121.7 (C-12), 143.5 (C-13), 203.7 (C-1'), 43.1 (C-2'), 70.4 (C-3'), 76.2 (C-4'), 64.7 (C-5').

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