ISOLATION OF ALKALOIDS FROM CULTURED HYBRID CELLS OF *RAUWOLFIA SERPENTINA* x *RHAZYA STRICTA*

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Two monoterpenoid indole alkaloids and four β-carbolines were isolated from a hybrid cell suspension culture generated from two Apocynaceous plants, *Rauwolfia serpentina* Benth. and *Rhazya stricta* Decaisne. This indicates that the function of alkaloid biosynthesis is retained after hybrid formation and that alkaloids not previously detected in the parental plants or cell cultures are formed.

KEY WORDS hybrid cell; *Rauwolfia serpentina*; *Rhazya stricta*; Apocynaceae; indole alkaloid

Hybrid cells, obtained by the fusion of different plant protoplasts, are expected to provide new biological sources for the creation and production of novel, biologically active natural products. We recently succeeded in stimulating the proliferation of hybrid cells[1] prepared from cell cultures of two different Apocynaceous plants, *Rauwolfia serpentina* Benth. ex Kurz[2] and *Rhazya stricta* Decaisne.[2] *R. serpentina* from tropical and subtropical zones has played an important role as a rich source of useful medicines, such as the antiarrhythmic alkaoid ajmaline and the antihypertensive compound reserpine. *R. stricta*, native to the northwestern Indian subcontinent, is reputed in Indian folk medicine to be a bitter tonic and a cure for chronic rheumatism. During our studies of secondary metabolites produced by plant cell cultures we investigated the chemical constituents in cultured hybrid cell suspensions of *R. serpentina* x *R. stricta*.[3] This paper describes the isolation and structure elucidation of alkaloidal constituents from cultured hybrid cells.

Freeze-dried hybrid cells (100 g) cultured for 14 days with AP-medium[4] were extracted with hot MeOH to give the extract (44 g). A basic fraction (169 mg) from the MeOH extract was subjected to silica gel column chromatography and then to medium-pressure liquid column chromatography to provide four β-carboline compounds (Compounds A-D) and two monoterpenoid indole alkaloids (Compounds E, F).

Compounds A (**1**) and B (**2**) exhibited characteristic UV absorptions, suggesting the presence of a β-carboline skeleton with a carbonyl function on the C-1 position. These compounds were identified as 1-acetyl-β-carboline (**1**) [5] and 1-methoxycarbonyl-β-carboline (**2**) [6] by comparison of the ^{1}H-NMR and HR-FABMS data with those of reported materials.

Compound C (**3**) displayed the typical UV absorptions of the β-carboline chromophore and was identified as β-carboline (**3**) by comparison of the spectroscopic data.[7]

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A new compound, Compound D (**4**), [8] was obtained as an amorphous powder (2 mg); UV absorptions of **4** at 217, 234 (sh), 243, 251, 260, 284, 307 and 380 nm indicated the presence of a β-carboline nucleus having a carbonyl function on the C-1 position.[5] In the ^{1}H-NMR of **4**, six aromatic protons due to a β-carboline skeleton and six aliphatic protons were observed. The ^{13}C-NMR spectrum of **4** indicated the presence of a carbonyl carbon (δ 203.6), eleven aromatic carbons, two aliphatic methylene (δ 43.1, δ 64.7) and two methine (δ 70.3, δ 76.2) carbons. The HR-MS displayed the molecular ion at *m/z* 300.1102 ($C_{16}H_{16}N_2O_4$), indicating that **4** had three aliphatic hydroxyl groups. The assignments of protons and carbons were confirmed by decoupling experiments, distortionless enhancement by polarization transfer (DEPT), ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY and ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) NMR spectra. Decoupling experiments, ^{1}H-^{1}H COSY, and ^{13}C-^{1}H COSY indicated the presence of the partial structure (**5**). The cross peak between δ_C 203.6 and δ_H 3.5 in the HMBC spectrum demonstrated that the aliphatic side chain was attached to the carbonyl carbon. Observation of a fragment ion at *m/z* 239 [M-(CHOH)-(CH_2OH)] in the EI-MS suggested that the molecule has a terminal CH(OH)-CH_2OH residue on the side chain. On the basis of the above observations, the structure of Compound D was deduced to be **4**. Determination of the structure of Compound D, including the relative and absolute configurations by means of total synthesis, will be presented in a subsequent paper.

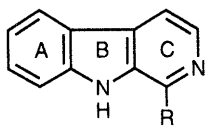
Compound E (**6**) exhibited typical UV absorptions of an indole chromophore. The EI-MS showed the molecular ion at *m/z* 354. The ^{1}H-NMR spectrum showed that **6** possessed an indole skeleton, an ethylidene side chain (δ 5.64, ddd, J = 7.0, 7.0, 7.0 Hz, δ 1.69, dd, J = 6.8, 1.7 Hz) and a methoxyl group (δ 3.82, s). From these observations Compound E was deduced to be an isositsirikine-related compound having a *Corynanthe*-type skeleton. Cross peaks between 19-H and 21-H and between 18-Me and 15-H in the NOESY experiment suggested 19,20-*E* geometry. On the basis of the comparison of ^{1}H-NMR data with reported data, Compound E was identified as 16(*R*)-19,20(*E*)-isositsirikine (**6**).[9,10] From the original plant of *R. stricta*, isositsirikine-type compounds rhazimanine,[11] bhimberine[12] and 16(*S*)-19,20(*Z*)-isositsirikine[13] were isolated.

The UV spectrum of Compound F (**7**) revealed the presence of an indole skeleton and an acrylic ester chromophore. The FAB-MS showed the protonated molecular ion at *m/z* 589. ^{1}H- and ^{13}C-NMR spectra of **7** were similar to those of 5(*S*)-5-carboxystrictosidine (**8**),[14,15] except for the presence of two carbomethoxyl signals in **7**. 5(*S*)-5-carboxystrictosidine (**8**), which was obtained by the condensation of secologanin with L-tryptophan, was treated with diazomethane to give Compound F. Consequently Compound F was identified as 5(*S*)-5-carbomethoxystrictosidine (**7**).

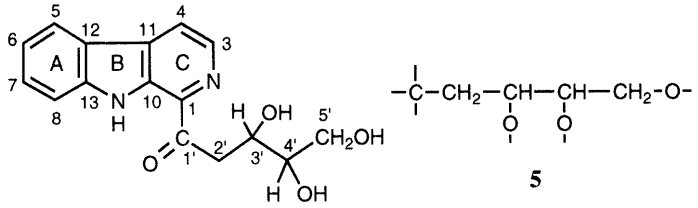
In conclusion, from cultured hybrid cells of *R. serpentina* x *R. stricta*, 1-acetyl-β-carboline, 1-methoxycarbonyl-β-carboline, β-carboline, 16(*R*)-19,20(*E*)-isositsirikine, and 5(*S*)-5-carbomethoxystrictosidine were isolated together with the novel indole alkaloid **4**. These compounds, except for 16(*R*)-19,20(*E*)-isositsirikine, have not been found from either plant. The alkaloid **4** could also be isolated from the freshly prepared and sterilized culture medium. Therefore it might not be biosynthesized but could be an artefact formed by condensation of tryptophan and a sugar unit such as glucose. L-Tryptophan is in fact a constituent of the nutrition medium used and glucose would derive from sucrose by hydrolysis during the sterilization process of the nutrition medium.

The above results have clearly demonstrated that cultured hybrid cells maintain the function of monoterpenoid indole alkaloid production which the parent plants and cells possessed. It is,

however, also important to note that cultured hybrid cells produce several kinds of alkaloids that have not been isolated from either of the original plants or cell cultures and therefore provide interesting cell systems for future biosynthetic research.

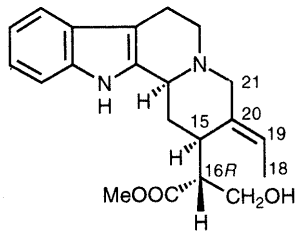


R=COMe : 1-Acetyl-β-carboline (**1**)
R=COOMe : 1-Methoxycarbonyl-β-carboline (**2**)
R=H : β-Carboline (**3**)

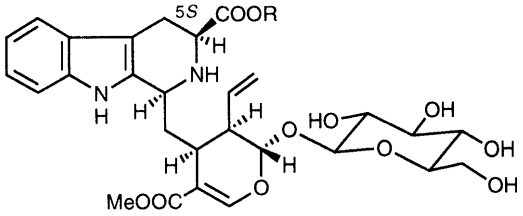


Compound D (**4**)

5



16(*R*)-19, 20(*E*)-Isositsirikine (**6**)



R=Me: 5(*S*)-5-Carbomethoxystrictosidine (**7**)
R=H: 5(*S*)-5-Carboxystrictosidine (**8**)

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REFERENCES AND NOTES

1) Kostenyuk I., Lubaretz O., Borisyuk N., Voronin V., Stöckigt J., Gleba Y., *Theor. Appl. Genet.*, **82**, 713-716 (1991).
2) Southon I. W., Buckingham J., "Dictionary of Alkaloids", Chapman and Hall, London, **1989**.
3) a) Pawelka K.-H., Stöckigt J., *Z. Naturforsch,* **41c**, 385-390. (1986); b) Ruyter C. M., Schübel H., Stöckigt J., *Z. Naturforsch,* **43c**, 479-484 (1988) and references cited therein; c) Polz L., Stöckigt J., Takayama H., Uchida N., Aimi N., Sakai S., *Tetrahedron Lett.*, **31**, 6693-6696 (1990); d) Aimi N., Uchida N., Ohya N., Hosokawa H., Takayama H., Sakai S., Mendonza L. A., Polz L., Stöckigt J., *Tetrahedron Lett.*, **32**, 4949-4952 (1991); e) Endreß S., Suda S., Takayama H., Kitajima M., Aimi N., Sakai, S., Stöckigt, J., *Phytochemistry*, **32**, 725-730 (1993) and references cited therein; f) Aimi, N., Uchida N., Ohya N., Sakai S., Mendonza L. A., Obitz P., Stöckigt J., *Heterocycles*, **38**, 2411-2414 (1994); g) Obitz P., Stöckigt J., Mendonza L. A., Aimi N., Sakai S., "Alkaloids: Chemical and Biological Perspectives", Vol. 9, ed. by Pelletier S. W., Pergamon Press, 1994, pp. 235-245.
4) Schübel, H., Ruyter, C. M., Stöckigt, J., *Phytochemistry*, **28**, 491-494 (1989).
5) Ohmoto T., Koike K., *Chem. Pharm. Bull.*, **30**, 1204-1209 (1982).
6) Kondo Y., Takemoto T., *Chem. Pharm. Bull.*, **21**, 837-839 (1973).
7) Atta-ur-Rahman, Hasan S., Qulbi M. R., *Planta Medica,* **1985**, 287.
8) $[\alpha]_D^{23}$ -39° (*c* 0.048, MeOH). HR-MS *m/z*: 300.1102 (Calcd for $C_{16}H_{16}N_2O_4$: 300.1110). EI-MS *m/z* (%): 300 (M^+, 26), 282 (23), 264 (82), 247 (38), 239 (33), 221 (34), 211 (100), 182 (30), 168 (93). ^{1}H-NMR (500 MHz, CD_3OD) δ: 3.60-3.68 (m, 4H, 2'-H_2, 4'-, 5'-H), 3.82 (dd, 1H, *J* = 10.7, 3.3 Hz, 5'-H), 4.34 (m, 1H, 3'-H), 7.31 (ddd, 1H, *J* = 8.0, 8.0, 1.0 Hz, 6-H), 7.59 (ddd, 1H, *J* = 8.3, 7.2, 1.1 Hz, 7-H), 7.70 (d, 1H, *J* = 8.3 Hz, 8-H), 8.21 (dd, 1H, *J* = 8.0, 1.0 Hz, 5-H), 8.30 (d, 1H, *J* = 4.8 Hz, 4-H), 8.46 (d, 1H, *J* = 5.2 Hz, 3-H). ^{13}C-NMR (125 MHz, CD_3OD) δ: 137.3 (C-1), 138.5 (C-3), 120.1 (C-4), 122.7 (C-5), 121.59 (C-6), 130.3 (C-7), 113.4 (C-8), 136.3 (C-10), 133.3 (C-11), 121.62 (C-12), 143.4 (C-13), 203.6 (C-1'), 43.1 (C-2'), 70.3 (C-3'), 76.2 (C-4'), 64.7 (C-5').
9) Kan C., Kan S-K., Lounasmaa M., Husson H.-P., *Acta Chem. Scand.*, **B. 35**, 269 (1981).
10) Kohl W., Witte B., Sheldrick W. S., Höfle G., *Planta Medica*, **50**, 242-244 (1984) .
11) Atta-ur-Rahman, Malik S., Habib-ur-Rehman, *Phytochemistry*, **25**, 1731-1733 (1986).
12) Atta-ur-Rahman, Habib-ur-Rehman, Malik S., *Heterocycles*, **24**, 703-709. (1986). Recently the structures of rhazimanine and bhimberine were revised to be 16(*R*)-19,20(*E*)-isositsirikine. Lounasmaa M., Jokela R., Hanhinen P., Miettinen J., Salo J., *J. Nat. Prod.*, **58**, 131-133 (1995).
13) Mukhopadhyay S., El-Sayed A., Handy G. A., Cordell G. A., *J. Nat. Prod.*,**46**, 409-413 (1983).
14) Ferrari F., Messana I., Botta B., De Mello J. F., *J. Nat. Prod.*, **49**, 1150-1151 (1986).
15) Aimi N., Seki H., Sakai S., *Chem. Pharm. Bull.*, **40**, 2588-2590 (1992).

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