13 Kulanthaivel, P. and Pelletier, S W. (1987) *Tetrahedron Letters* **28**, 3883.

14. Pelletier, S W, Chokshi, H P and Desai, H K (1986) *J. Nat Prod.* **49**, 892

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15β-HYDROXYVINCADIFFORMINE, AN ALKALOID FROM THE LEAVES OF *RHAZYA STRICTA*

ATTA-UR-RAHMAN*, TALAT FATIMA and SAJIDA KHANUM

H. E. J Research Institute of Chemistry, University of Karachi, Karachi 32, Pakistan

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Key Word Index—*Rhazya stricta*, Apocynaceae, alkaloid; 15β-hydroxyvincadifformine, homodecoupling; ^{13}C NMR, DEPT

Abstract—A new alkaloid, 15β-hydroxyvincadifformine has been isolated from the leaves of *R. stricta*.

INTRODUCTION

Rhazya stricta (Decaisne) is a small glabrous erect shrub, abundantly distributed in Pakistan [1–3]. The plant is well known in the indigenous system of medicine for the treatment of various diseases [4–7]. Extracts of *R. stricta* showed anti-cancer and antineoplastic activity [8–11] We have previously reported a number of new alkaloids from the plant [4, 12–21]. In continuation of our studies on the isolation and structure elucidation of new chemical constituents from the leaves of *Rhazya stricta*, we have isolated a new alkaloid, 15β-hydroxyvincadifformine (**1**), the structure of which has been established by spectroscopic studies.

RESULTS AND DISCUSSION

The crude alkaloidal material obtained from the ethanolic extract of the fresh leaves of *Rhazya stricta* by conventional procedures [4, 5] was subjected to column chromatography for preliminary fractionation The fraction obtained on elution with petrol–chloroform (1:3) afforded a mixture of alkaloids. This mixture was subjected to repeated chromatographic purification on silica gel (Merck, GF-254) in petrol–chloroform–methanol (14:5.1). The faster moving alkaloid was obtained as a pale yellow amorphous material and gave a UV spectrum characteristic of the anilinoacrylate system. The IR spectrum showed peaks at 3420 (–NH), 3400 (OH) and 1690 cm^{-1} (conjugated ester· –N–C=C–CO_2Me). The HRMS afforded M at 354 1943 ($C_{21}H_{26}N_2O_3$), indicating the presence of 10 double bond equivalents in the molecule and a fragmentation characteristic of the Aspidosperma skeleton. The ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) showed the presence of 26 protons. A three-proton singlet at δ3.75 was assigned to the ester methyl protons. Another three proton singlet at δ0.67 ($J_{18,19}$ = 7.5 Hz) was assigned to the methyl protons of the ethyl group. Two quartets resonated at δ0.96 ($J_{19\alpha,18}$ = 7.5 Hz) and δ1.11 ($J_{19\beta,18}$ = 7.5 Hz) for the C-19 methylene protons of the ethyl group, their separate chemical shifts being on account of their prochiral nature. Such a differentiation between the two methylene protons of the ethyl group has previously been observed in vindoline [22] and bannucine [23]. A multiplet for C-15α H appeared at δ3.74 Other chemical shift assignments for 15β-hydroxyvincadifformine are shown in Table 1.

Two dimensional NMR measurements (COSY-45, 2D *J*-resolved) were carried out to verify the assignments. The coupling interactions were established through COSY-45 spectrum while the multiplicities of the overlapping proton signals were determined from the 2D *J*-resolved spectrum. The assignments for the C-18 methyl protons at δ0 66 could thus be confirmed from the COSY-45 spectrum, which showed strong cross peaks with the signals at δ0.99 for C-19Hα and at δ1.11 for C-19H β protons. The signal at δ3.74 (C-15H α) showed strong cross peaks with the signal at δ2.01 (C-14α), while the signal at δ2.23 (C-5) showed cross peaks with the signal at δ1.74 C-6 protons The COSY-45 spectrum of 15β-hydroxyvincadifformine (**1**) is presented in Fig. 1 with the important interactions indicated.

The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz, DEPT) of the compound showed a downfield signal at δ169.30 for the ester carbonyl group. Another downfield quaternary signal at δ167.23 was assigned to the C-2 carbon atom. A downfield signal for the hydroxyl-bearing C-15 methine carbon resonated at δ70.51, its downfield chemical shift

Table 1 ^{1}H NMR spectral data of 15β-hydroxyvincadifformine (1)

H	ppm	
3Hα	2.56 *dd*	$J_{3\alpha,3\beta}$ = 15 1 Hz, $J_{3\alpha,14\alpha}$ = 8 0 Hz
3Hβ	3.14 *ddd*	$J_{3\beta,15\alpha}$ = 5 5 Hz, $J_{3\beta,14\beta}$ = 9 8 Hz, $J_{3\beta,3\alpha}$ = 16 3 Hz
5Hα	2.74 *m*	
5Hβ	2 23 *m*	
6Hα	2 11 *dd*	$J_{6\alpha,5\alpha}$ = 6 4 Hz, $J_{6\alpha,6\beta}$ = 11 5 Hz
6Hβ	1.73 *dd*	$J_{6\beta,6\alpha}$ = 11 55 Hz, $J_{6\beta,5\beta}$ = 4 3 Hz
9H	7 15 *d*	$J_{9,10}$ = 7 35 Hz
10H	6 85 *t*	$J_{10,11}$ = 7 3 Hz, $J_{10,9}$ = 7 25 Hz
11H	7 11 *t*	$J_{11,12}$ = 7.2 Hz, $J_{11,10}$ = 7 3 Hz
12H	6 80 *d*	$J_{12\ 11}$ = 7.2 Hz
14Hβ	1 80 *m*	
14Hα	2 00 *m*	
15Hα	3 74 *m*	
17Hβ	2 64 *d*	$J_{17\beta,17\alpha}$ = 15.0 Hz
17Hα	2 92 *d*	$J_{17\alpha,17\beta}$ = 14 67 Hz
18H	0 67 *t*	$J_{18,19}$ = 7 5 Hz
19Hα	0 96 *q*	$J_{19\alpha,18}$ = 7 5 Hz
19Hβ	1 11 *q*	$J_{19\beta,18}$ = 7 35 Hz
21Hα	3 96 *s*	
OCH_3	3 75 *s*	
N–H	8 93 *s*	

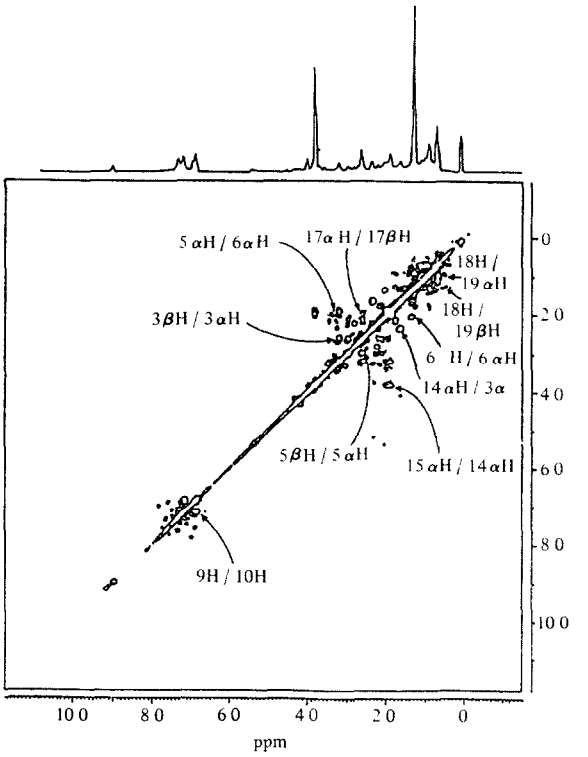


Fig. 1

indicating the presence of 15β-hydroxyl configuration, (in 15α-hydroxy compound, the signal occurs at δ67.88 [24]). The C-18 methyl carbon atom appeared at δ8.45. Other ^{13}C-NMR chemical shift assignments are presented in Table 2. This data led to structure (1), corresponding to 15β-hydroxyvincadifformine, for the substance. 15β-Hydroxyvincadifformine is known synthetically as an intermediate in the synthesis of tabersonine [24]

Table 2. ^{13}C-NMR spectral data of 15β-hydroxyvincadifformine (1)

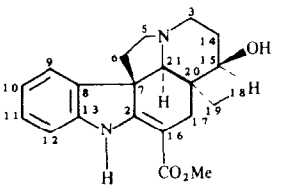
C	ppm	multiplicity
2	167.23	*s*
3	51 29	*t*
5	47 43[a]	*t*
6	45 43[a]	*t*
7	55 57	*s*
8	137 50	*s*
9	121.20	*d*
10	120 70	*d*
11	127 70	*d*
12	109 47	*d*
13	143 35	*s*
14	31 38	*t*
15	70 51	*d*
16	92 97	*s*
17	22 58	*t*
18	8 45	*q*
19	26 57	*t*
20	43 71	*s*
21	74 71	*d*
COOMe	50 98	*q*
COOMe	169 30	*s*

[a] Assignments may be interchanged.

EXPERIMENTAL

General. UV IR The ^{1}H NMR spectra were recorded at 300 MHz and the ^{13}C NMR spectra at 75 MHz TLC experiments were performed on silica gel (GF-254, 0 2 mm) precoated plates Merck)

The EtOH extracts of the fresh leaves (95 kg) of *Rhazya stricta* were concentrated to a gum which was dissolved in 10% HOAc (11) the non-alkaloidal part was removed by extraction with EtOAc (25 l), the aq acidic soln basified with aq NH_3 (500 ml) to pH 11 and extracted with EtOAc (36 l) to afford the crude alkaloids (350 g) This alkaloidal material was subjected to CC (alkaloid silica gel, 1 40) Elution with increasing polarities of C_6H_6, petrol, $CHCl_3$, EtOAc and MeOH afforded several fractions. A fraction obtained on elution with petrol–$CHCl_3$ (1 3) was found to be a mixture of alkaloids, (5 g) This alkaloidal mixture was further purified on repeated column chromato-



15 β- Hydroxyvincadifformine (1)

graphy over silica gel. Elution with $CHCl_3$–MeOH (9·1) afforded a partly purified mixture of alkaloids (30 mg) Further purification yielded the new alkaloid, 15β-hydroxyvincadifformine (**1**) (12 mg). 15β-hydroxyvincadifformine (**1**) gave a light orange colouration with Dragendorff's reagent and deep blue colouration with ceric sulphate spray

15β-Hydroxyvincadifformine (**1**) $[\alpha]_D$ $CHCl_3 = +240.57°$ UV (MeOH). λ_{max} nm, 205, (log ε 3.90) 224 (log ε 3.85), 296 (log ε 3.99), 327 (log ε 4.17), λ_{min} nm, 218 (log ε 4 58), 260 (log ε 3.52), 305 (log ε 3.20) IR ($CHCl_3$) ν_{max} cm^{-1}, 3420 (–NH), 3400 (–OH) 1690 (ester C=O) HRMS· Observed MS rel, 354 (20%, $C_{21}H_{26}N_2O_3$), 336 1837 (6%, $C_{21}H_{24}N_2O_2$), 323 1759 (5%, $C_{20}H_{23}N_2O_2$), 141 1153 (25% $C_8H_{15}NO$), 140 1075 (100%, $C_8H_{14}NO$). ^1H NMR ($CDCl_3$, 300 MHz) Table 1 ^{13}C NMR ($CDCl_3$, 75 MHz, DEPT)· Table 2

REFERENCES

1 Hooker, J D and Jackson, B D. (1865) *Index Kewensis* **4**, 705
2. Bisset, N G (1958) *Ann Bogor* **3**, 105.
3 Atta-ur-Rahman and Fatima, K. (1982) *J. Chem Soc Pak.* **4**, 121
4 Ahmad, Y., Fatima, K., Quesne, P W. Le and Atta-ur-Rahman (1983) *Phytochemistry* **22**, 1017.
5. Chopra, R N, Nayar, S. L and Chopra, I. C. (1956) *A Glossary of Indian Medicinal Plants*, p. 212. C S I R, New Delhi.
6. Dymock, W., Warden, C J. H. and Hooper, D. H. (1983) *Pharmacographia Indica* Vol 3, p. 3911. Kegan, Paul, Trench, Trubner and Company, London
7. Watt, G. (1892) *A Dictionary of the Economic Products of India.* W. H. Allen, London.
8. Hooper, D (1906) *Pharm. J.* **77**, 258.
9. Siddiqui, S and Bukhari, A Q. S (1972) *Nature* **235**, 393.
10. Mukhopadhyay, S., Handy, G. A., Funayama, S. and Cordell, G A (1981) *J. Nat. Prod.* **44**, 696
11. Mukhopadhyay, S., Sayed, A. El, Handy, G. A. and Cordell, G. A. (1983) *J. Nat Prod.* **46**, 409.
12. Atta-ur-Rahman and Khanum, S (1984) *Phytochemistry* **23**, 709.
13 Atta-ur-Rahman and Khanum, S (1984) *Heterocycles* **22**, 2183
14. Atta-ur-Rahman, Habib-ur-Rehman and Malik, S. (1986) *Heterocycles* **24**, 703
15 Atta-ur-Rahman, Malik, S and Habin-ur-Rehman (1986) *Phytochemistry* **25**, 1731
16. Atta-ur-Rahman and Khanum, S (1984) *Tetrahedron Letters* **25**, 3913
17 Atta-ur-Rahman and Khanum, S (1984) *Phytochemistry* **24**, 2023
18. Atta-ur-Rahman and Zaman, K. (1984) *Heterocycles* **22**, 2023
19. Atta-ur-Rahman and Zaman, K. (1986) *Phytochemistry* **25**, 1779.
20 Atta-ur-Rahman and Khanum, S. (1985) *Heterocycles* **26**, 405.
21 Atta-ur-Rahman, Khanum, S., Fatima, K, Ahmad, Y. and Badar, Y (1987) *Z. Naturforsch.* **42** 91.
22. Hunter, B. K., Hall, L. D. and Sanders, J. K. M (1983), *J. Chem. Soc., Perkin Trans. I.* 657.
23. Atta-ur-Rahman, Ali, A. and Choudhary, M. I. (1986), *J. Chem. Soc., Perkin Trans. I.* 923
24. Kuehne, M E., Bornmann, W. G., Earley, W. G. and Marko, I. (1986) *J Org Chem* **51**, 2913.