8. Caprasse, M., Tavernier, D., Anteunis, M. and Angenot, L. (1984) *Planta Med.* **50**, 27.
9. Caprasse, M., Angenot, L., Tavernier, D. and Anteunis, M. (1984) *Planta Med.* **50**, 131.
10. Ito, Y. (1986) *CRC Critical Rev. Anal. Chem.* **17**, 65.
11. Khan, Z. M., Hesse, M. and Schmid, H. (1965) *Helv. Chim. Acta* **48**, 8, 1957.
12. Jordan, W. and Scheuer, P. J. (1965) *Tetrahedron* **21**, 3731.
13. Coune, C., Tits, M. and Angenot, L. (1982) *J. Pharm. Belg.* **37**, 3, 189.
14. Massiot, G., Thepenier, Ph., Jacquier, M. J., Le Men-Olivier, L., Verpoorte, R. and Delaude, C. (1987) *Phytochemistry* **26**, 10, 2839.
15. Seguin, E., Hotellier, F., Koch, M. and Sevenet, Th. (1984) *J. Nat. Prod.* **47**, 4, 687.
16. Sakai, S., Aimi, N., Takahashi, K., Kitagawa, M., Yamaguchi, K. and Haginiwa, J. (1974) *Yakugaku Zasshi* **94**, 1274.
17. Toth, G., Hetenyi, F., Clauder, O. and Kajtar, M. (1978) *Liebigs Ann. Chem.* 1096.
18. Wenkert, E., Bindra, J. S., Chang, Ch. J., Cochran, D. W. and Schell, F. M. (1974) *Accounts Chem. Res.* **7**, 46.
19. Gribble, G. W., Nelson, R. B., Johnson, J. L. and Levy, G. C. (1975) *J. Org. Chem.* **40**, 25, 3720.
20. Angenot, L., Coune, C. and Tits, M. (1978) *J. Pharm. Belg.* **33**, 11.

Phytochemistry, Vol. 27, No. 6, pp. 1926–1928, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00
Pergamon Press plc.

STUDIES ON THE ALKALOIDS OF *RHAZYA STRICTA*

ATTA-UR-RAHMAN* and KHURSHID ZAMAN

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

(*Revised received* 6 *October* 1987)

Key Word Index—*Rhazya stricta*, Apocynaceae, dimeric indole alkaloids, secamine, decarbomethoxytetrahydrosecamine, 1,2-dehydroaspidospermidine *N*-oxide.

Abstract—Two new dimeric indole alkaloids, 16*S*,16′-decarbomethoxytetrahydrosecamine (**1**) and 16*R*,16′-decarbomethoxytetrahydrosecamine (**2**) have been isolated from the roots of *Rhazya stricta*. The structures of the two isomers have been established through spectroscopic studies. The ^{13}C NMR spectrum of 1,2-dehydroaspidospermidine-*N*-oxide (**3**) is also presented.

INTRODUCTION

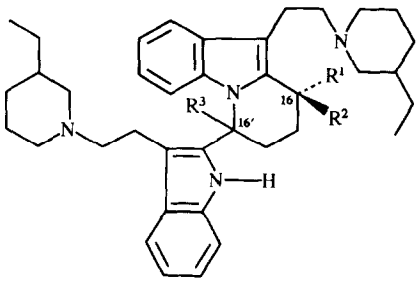
Rhazya stricta (Apocynaceae) is widely distributed in Pakistan and has been used for the treatment of various ailments [1–6]. The anticancer activity of some of its alkaloids is also reported [7–9]. We have previously reported a number of new alkaloids from the plant [10–13] including didemethoxycarbonyltetrahydrosecamine from the roots [13] which was found to be cytotoxic against Eagles KB carcinoma of the nasopharynx in cell cultures [8]. '16-Decarbomethoxytetrahydrosecamine' was previously reported from the roots of *Amsonia tabernaemontana* and from the rootbark of *Aspidosperma excelsum* but it was not determined whether the isolated compound possessed 16*S* or 16*R*-stereochemistry [14, 15]. In this communication we report the isolation and structure of both the isomeric biologically interesting dimeric alkaloids 16*S*,16′-decarbomethoxytetrahydrosecamine (**1**) and 16*R*,16′-decarbomethoxytetrahydrosecamine (**2**), together with ^{13}C NMR studies on 1,2-dehydroaspidospermidine-*N*-oxide (**3**).

RESULTS AND DISCUSSION

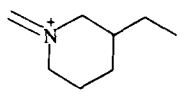
The alkaloids 16*S*,16′-decarbomethoxytetrahydrosecamine (**1**) $[\alpha]_D = 0°$ and 16*R*,16′-decarbomethoxytetrahydrosecamine (**2**) $[\alpha]_D = 0°$, were obtained after separation of the alkaloidal fractions on the basis of differential basicities and preparative TLC. The UV spectra of both were identical to those reported for secamines [6, 8, 13, 14] showing λ_{max}(MeOH) 220, 283, 291. They also possessed virtually identical IR spectra and gave absorptions at 3650 (NH), 2900, 1720 (satd ester C=O), 1600, 1340 and 1010 cm^{-1}.

The mass spectrum of each afforded the molecular ion peak at *m/z* 622 with the exact masses of (**1**) and (**2**) at 622.4576 ($C_{40}H_{54}N_4O_2$) and 622.4273 ($C_{40}H_{54}N_4O_2$)

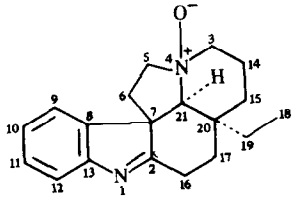
*In our earlier publication on the structure of didemethoxycarbonyltetrahydrosecamine, we have assigned the C-16′H at δ4.04. An examination of the spectra of the repurified material leads us to reverse the assignments as follow: δ4.01 (C-16H), δ5.33 (C-16′H) [13].



1 $R^1 = R^3 = H$, $R^2 = CO_2Me$
2 $R^1 = CO_2Me$, $R^2 = R^3 = H$



(a)



3

respectively. This showed the presence of 16 double bond equivalents. The molecular ion peaks were further confirmed by +ve FAB mass spectroscopy. Other fragments in the EIMS of (**1**) appeared at *m/z* 564.4186 ($C_{38}H_{52}N_4$, 4.32%), 496, 451.2985 ($C_{31}H_{37}N_3$), 313 (2.2%), 239 (1.5%), 168 (1.83%) and 126 ($C_8H_{16}N$, 100%). The mass spectral fragmentation pattern of (**2**) was almost identical to that of (**1**). The formulae of the ions were established by computer monitored high resolution mass measurements and confirmed by peak matching experiments. The most prominent feature of both mass spectra was strong base peaks at *m/z* 126 (a). This is characteristic of the tetrahydrosecamine system containing a saturated 3-ethylpiperidine ring.

The ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of (**1**) showed the presence of two overlapping triplets in the region δ0.85–0.92 (J = 7.3 Hz, 2 × CH_2Me) which were assigned to the C-18 and C-18′ methyl protons. When the spectrum is recorded in d_6-acetone the C-18 and C-18′ methyl protons were observed to resonate at δ0.75 and 0.87 respectively. A broad region of overlapping multiplets at δ1.59–3.22 was ascribed to the 32 methylenes and four methine protons. The most distinguishing feature of the ^{1}H NMR spectrum was the positions of the ester methyl protons. It has previously been shown in tetrahydrosecamine that when the two ester groups at C-16 and C-16′ are both β-oriented then the ester methyl protons resonate at δ3.72 and 3.75 [16]. In the corresponding 16-epimer the signal of the C-16 ester methyl is shifted upfield to δ3.63 while the C-16′ ester methyl protons remain relatively unaffected (δ3.74). Clearly inversion at C-16′ from the β isomer to the α isomer gives rise to a deshielding effect of δ0.11 ppm. Examination of the ^{1}H NMR spectrum of the two compounds showed that the ester methyl in (**1**) resonated at δ3.57 while in (**2**) it resonated at δ3.78. This provided strong evidence that in (**1**) the CO_2Me was β-oriented while in (**2**) the CO_2Me was α-oriented. Furthermore (**2**) contained low field 1H multiplets at δ4.01 and 5.24 which were assigned to the C-16 and C-16′ protons*. This ruled out the possibility of the CO_2Me group being present at C-16. The rest of the ^{1}H NMR spectrum of (**1**) was similar to that of (**2**). Both compounds were susceptible to interconversion on TLC.

As a consequence of these considerations, the two alkaloids have been assigned structures as 16*S*,16′-decarbomethoxytetrahydrosecamine (**1**) and 16*R*,16′-decarbomethoxytetrahydrosecamine (**2**). A ^{13}C NMR spectrum was obtained but the multiple signals in the broad band spectrum for many of the carbon atoms, presumably on account of conformational mobility, prevented unambiguous assignments. A similar doubling of signals has previously been reported in such compounds [15]. The ^{13}C NMR of 16*S*,16′-decarbomethoxytetrahydrosecamine (**1**) and 16*R*,16′-decarbomethoxytetrahydrosecamine (**2**) showed a signal at δ79 for C-16′. When the C-16H is in a β-configuration, this carbon is known to resonate at δ50.7, [15]. This suggests that the substance in hand has C-16H in an α-configuration. However no confirmatory evidence could be obtained in this respect, due to paucity of samples. The rather downfield chemical shift may be attributed to the deshielding caused by the carbonyl of the ester group at C-16. The alkaloids probably arise in the plant by hydrolysis and decarboxylation of tetrahydrosecamine at C-16′. They may possess cytotoxic activity and are to be tested against cancer.

1,2-Dehydroaspidospermidine *N*-oxide (**3**) has previously been reported and its characterization by spectroscopic methods described [11]. Attempted deoxygenation by reduction with phosphorus trichloride and by treatment with sodium borohydride led to the formation of 1,2-dehydroaspidospermidine along with an indolic base, the structure of which is under investigation. Alkaloid *N*-oxides are abundant in indole and indoline alkaloids, but their occurrence in the indolenine series is rare [17, 18]. Thus (**3**) is a new addition to the naturally occurring *N*-oxides in the indolenine series. The ^{13}C NMR spectrum however could not be recorded previously due to lack of sufficient material, We have now reisolated this compound and recorded its ^{13}C NMR spectrum. The chemical shifts of the aromatic carbons were assigned by comparison with the chemical shifts of other indolenine alkaloids [19]. The values are similar to those of raucaffricine [19] and perakine [19]. The amino methylene carbons C-3 and C-5 can be distinguished as the lowest field methyl signals at δ54.59 and δ52.04 respectively. The *N*-oxide oxygen was seen to induce a downfield shift of up to 2–4 ppm at C-3 and C-5 in comparison to the corresponding carbon atoms in (−)-vincadifformine [20]. Carbon-6 resonated at δ35.21 in 1,2-dehydroaspidospermidine *N*-oxide. This value is close to the corresponding reported values of 19-*S* vindolinine and 19-*R* vindolinine [21]. Carbon-21 (methine)

Table 1. ^{13}C NMR ($CDCl_3$, 75 MHz) Chemical shifts (ppm from TMS, multiplicities of signals were determined by DEPT measurements)

C	Values	Multiplicities
2	192.44	C
3	54.59	CH_2
5	52.04	CH_2
6	35.21	CH_2
7	*	C
8	*	C
9	121.00	CH
10	125.11	CH
11	128.65	CH
12	120.11	CH
13	154.54	C
14	22.09[a]	CH_2
15	33.27	CH_2
16	27.33	CH_2
17	23.78[a]	CH_2
18	7.30	Me
19	29.81	CH_2
20	36.59	CH
21	78.99	CH

*Signals were too weak to be recorded.
[a]Values are exchangeable.

resonated at δ78.99 suggesting the presence of an α-oriented proton on it [22, 23]. The α-configuration was also confirmed by ^1H NMR [24]. The ^{13}C NMR assignments of **3** are presented in Table 1, and agree with the structure and configuration of 1,2-dehydroaspidospermidine *N*-oxide. Polarisation transfer experiments allowed a verification of these assignments.

EXPERIMENTAL

The ^1H NMR spectra were recorded at 300 MHz in $CDCl_3$, UV in MeOH and IR in $CHCl_3$.

Isolation. The two alkaloids (**1**, **2**) were isolated from the crude alkaloidal fractions obtained by conventional procedures [25]. The alkaloidal extract was subjected to selective extractions with $CHCl_3$ at different pH values. The fraction obtained by extraction at pH 1 was subjected to CC on silica gel, elution being carried out with $CHCl_3$ and $CHCl_3$–MeOH mixtures of increasing polarity. This led to the isolation of several alkaloids. The fraction obtained on elution with 80% $CHCl_3$ 20% MeOH was evapd and subjected to prep. TLC on silica gel (GF-254) using EtOAc–isoPrOH–NH_4OH (6:3:1). This afforded two minor amorphous alkaloids (5 and 3 mg) respectively.

16S,16′-Decarbomethoxytetrahydrosecamine (**1**). $[\alpha]_D = 0°$ ($CHCl_3$), UV λ_{max}^{MeOH} nm: 220, 283, 291; λ_{min} nm: 250, 289; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 2900, 1720, 1600, 1340, 1010; found [M^+] 622.4576, $C_{40}H_{54}N_4O_2$ requires 622.4252; MS *m/z* (rel. int.): 622 (1.85), 578 (0.31), 509 (0.22), 369 (0.26), 313 (0.44), 239 (0.54), 126 (100), 69 (4.54); ^1H NMR (300 MHz, $CDCl_3$) δ0.9 (*t*, J = 7.2 Hz, 2 × CH_2Me), δ1.24–2.8 (*m*, 16 × CH_2, 4 × CH), δ3.57 (3H, *s*, CO_2Me), δ6.2–7.5 (*m*, Ar-H).

16R,16′-Decarbomethoxytetrahydrosecamine (**2**) $[\alpha]_D = 0°$ (in $CHCl_3$): UV λ_{max}^{MeOH} nm: 221, 284, 292; UV λ_{min}^{MeOH} nm: 250, 289, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2900, 1720, 1600: found [M^+] 622.4273; $C_{40}H_{54}N_4O_2$ requires 622.4252. MS *m/z* (rel. int.): 622 (12.43), 578 (9.19), 564 (4.32), 496, 451, 313 (2.20), 239 (1.59), 168 (1.83), 126 (100), 69 (24.63); ^1H NMR (300 MHz, $CDCl_3$) δ0.84 (*t*, 2 × CH_2Me), δ1.24–3.22 (*m*, 16 × CH_2, 4 × CH), δ3.78 (3H, *s*, CO_2Me), δ6.8–7.47 (Ar-H, *m*).

REFERENCES

1. Nadkarni's, K. M. (1976) *Indian Materia Medica* Vol. 1, p. 1056. Popular Prakashan Private, Bombay.
2. Kirtikar, K. R. and Basu, B. D. (1918) *Indian Medicinal Plants* Part II, p. 781. Indian Press, Allahabad.
3. Baquar, S. R. and Tasnif, M. (1967) *Medicinal Plants of Southern West Pakistan*, p. 43. Pakistan Council of Scientific and Industrial Research, Karachi.
4. Khan, A. A., Ashraf, M. and Nasreen Ali, M. (1979) *Pharmacognostic Studies of Selected Indigenous Plants of Pakistan*, p. 75, Pakistan Forest Institute, Peshawar.
5. Hooker, J. D. (1875) *Flora of British India* Vol. III, p. 640. Reeve, London.
6. Watt, G. (1892) *Dictionary of Economic Products of India* Part I, Vol. VI, p. 443, W. H. Aller, London.
7. Siddiqui, S. and Bukhari, A. Q. S. (1972) *Nature* **235**, 393.
8. Mukhopadhyay, S., Handy, G. A., Funayama, S. and Cordell, G. A. (1981) *J. Nat. Prod.* **44**, 696.
9. Mukhopadhyay, S., El-Sayed, A., Handy, G. A. and Cordell, G. A. (1983) *J. Nat. Prod.* **46**, 409.
10. Atta-ur-Rahman and Zaman, K. (1984) *Heterocycles* **22**, 2023.
11. Atta-ur-Rahman and Zaman, K. (1986) *Phytochemistry* **25**, 1779.
12. Atta-ur-Rahman and Zaman, K. (1986) *Planta Med.* 73.
13. Atta-ur-Rahman, Zaman, K., Malik, S. and Habib-ur-Rehman (1987) *J. Nat. Prod.* **49**, 1138.
14. Zsadon. B., Szilasi, M., Tamas, J. and Kaposi, P. (1975) *Phytochemistry* **14**, 1438.
15. Verpoorte, R., Kos-Kuyck, E., Tjin A Tosi, A., Ruigro, C. L. M., De Jong, G. and Baerheim Svendsen (1983) *Planta Med.* **48**, 283.
16. Cordell, G. A., Smith, G. F. and Smith, G. N. (1978) *J. Indian Chem. Soc.* **LV**, 1083.
17. Tunmann, P. and Wolf, D. (1969) *Z. Naturforsch.*, **24B**, 1665.
18. Atta-ur-Rahman and Khanum, S. (1984) *Phytochemistry* **23**, 709.
19. Ataullah Khan, M., Horn, H. and Voelter, W. (1982) *Z. Naturforsch.* **37b**, 494.
20. Wenkert, E., Cochran, D. W., Hagaman, E. W., Schell, F. M., Neuss, N., Katner, A. S., Potier, P., Kan, C., Plat, M., Koch, M., Mehri, H., Poisson, J., Kunesch, N. and Rolland, Y. (1973) *J. Am. Chem. Soc.* **95**, 4990.
21. Atta-ur-Rahman, Malik, S. and Albert, K. (1986) *Z. Naturforsch.* **41b**, 386.
22. Ahond, A., Janot, M. M., Langlois, N. Lukacs, G. and Potier, P., Neuss, N., Plat, M., Le Men, J., Hagaman, E. W. and Wenkert, E. (1974) *J. Am. Chem. Soc.* **96**, 633.
23. Rasoanaivo, P. and Lukacs, G. (1976) *J. Org. Chem.* **41**, 376.
24. Gorman, M., Neuss, N. and Biemann, K. (1962) *J. Am. Chem. Soc.* **84**, 1058.
25. Harborne, J. B. (1973) *Phytochemical Methods*, p. 6. Chapman & Hall, London.