

ALKALOIDS FROM THE HALLUCINOGENIC PLANT *VIOLA SEBIFERA*

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Key Word Index—*Viola sebifera*; Myristicaceae; hallucinogenic plant; alkaloids; *N*-methyl-*N*-acetyltryptamine; *N*-methyl-*N*-formyltryptamine; 2-methyl-1,2,3,4-tetrahydro- β -carboline; *N,N*-dimethyltryptamine; *N,N*-dimethyltryptamine-*N*-oxide; *N*-monomethyltryptamine.

Abstract—Bark of *Viola sebifera* used in the preparation of hallucinogenic snuffs and drinks in Venezuela has yielded *N*-methyl-*N*-formyltryptamine and *N*-methyl-*N*-acetyltryptamine, which exist in two rotameric forms reflecting hindered rotation around the carbon–nitrogen bond of the amide function. They were detected by HPLC as well as NMR. 2-Methyl-1,2,3,4-tetrahydro- β -carboline, *N,N*-dimethyltryptamine, its oxide and *N*-monomethyltryptamine were also identified.

INTRODUCTION

The principal alkaloid of the bark of *Viola sebifera*, a well-known psychotomimetic, has been shown to be *N,N*-dimethyltryptamine (DMT). 5-Hydroxy-DMT, 5-methoxy-DMT and 2-methyl-1,2,3,4-tetrahydro- β -carboline were also present but in small amounts [1]. We have now undertaken an additional study of this plant with particular reference to the characterization of its minor alkaloids.

RESULTS AND DISCUSSION

The bark of *V. sebifera* was extracted with methanol. After removal of the solvent, the crude alkaloids were extracted into chloroform by conventional partition procedures and chromatographed on silica gel by gradient elution using chloroform and methanol.

The first alkaloid thus obtained was eluted with chloroform alone. It was a non-crystalline base which gave an orange-red colour with Dragendorff's reagent, a blue colour with Ehrlich's reagent on TLC and UV absorption at 287.5, 277, 271 and 237.5 nm suggesting an

indole structure unsubstituted in the 2-position. The molecular formula determined by high resolution mass spectrometry was $C_{12}H_{14}N_2O$. In the IR spectrum, broad peaks at 3250 and 1645 cm^{-1} indicated indole NH and CO, respectively. 1H (200 MHz) and ^{13}C (50 MHz) NMR spectra (deuteriochloroform) were consistent with those of *N*-methyl-*N*-formyltryptamine (MFT) [2, 3], which was substantiated by the fragmentation pattern observed in the mass spectrum. A further study of the 1H NMR spectrum allowed us to assign structures to the rotamers. The smaller of the paired signals for *N*-methyl (δ 2.88) obtained in deuteriochloroform at 25° was shifted to δ 1.97 in deuterobenzene at 25°. The larger one (δ 2.93) in deuteriochloroform was shifted to δ 2.52 in deuterobenzene. The smaller signals for *N*-CH₂ (δ 3.70) and the larger (δ 3.54) in deuteriochloroform shifted upfield in deuterobenzene (δ 3.24 and 2.82, respectively) (Table 1). Thus, these signals indicate the structures **1a** and **1b** for the respective rotamers since alkyl groups with greater shifts upfield in deuterobenzene would have a *trans* orientation with respect to the carbonyl oxygen [4].

Two peaks for the rotamers (*R*_s: smaller, 21.4 min; larger, 26.8 min) were detected by HPLC on a silica gel

Table 1. 1H NMR chemical shifts in two amides and their rotamer ratio in deuteriochloroform and deuterobenzene at 25°

Compound	Structure	Group	Deutero- chloroform (δ)	Ratio (%)	Deutero- benzene (δ)	Ratio (%)
<i>N</i> -Methyl- <i>N</i> -formyltryptamine	1a	N-CH ₃	2.88	34.2	1.97	36.6
		N-CH ₂	3.70		3.24	
	1b	N-CH ₃	2.93	65.8	2.52	63.4
		N-CH ₂	3.54		2.82	
<i>N</i> -Methyl- <i>N</i> -acetyltryptamine	2a	N-CH ₃	2.99	51.2	2.12	57.4
		N-CH ₂	3.61		3.60	
	2b	N-CH ₃	2.92	48.8	2.72	42.6
		N-CH ₂	3.71		2.94	

column. However, no attempt was made to isolate them preparatively by HPLC because their rate of rotation was not expected to be slow enough to permit it at room temperature [5].

The following compound eluted with chloroform-methanol (24:1) and purified by CC and TLC was a resinous substance of formula $C_{13}H_{16}N_2O$, determined by high resolution mass spectrometry, and gave an orange-red colour with Dragendorff's reagent and a blue colour with Ehrlich's reagent on TLC. This alkaloid had UV absorption at 287.5, 277, 276 and 236 nm suggesting an indole structure unsubstituted in the 2-position and a broad peak at 3500 cm^{-1} in the IR spectrum indicating an indole NH. This compound was very similar to MFT, having paired signals on ^1H NMR: two for the acetyl methyl protons (δ 2.10 and 1.88) for the former were detected instead of two for the formyl protons for the latter. The rest of the paired signals were assigned as follows: δ 2.99 and 2.92 for *N*-methyl protons, δ 3.04, 3.61 and 3.04, 3.71 for the two carbon chain, δ 7.01 and 7.08 for H-2 on a 3-substituted indole; δ 7.17, 7.25 and δ 7.15, 7.23 for H-5 and H-6 or H-6 and H-5; δ 7.41 and 7.39 for H-4; δ 7.61 and 7.69 for H-7; δ 8.14 and 8.06 for NH. These data, in conjunction with the molecular formula implied the presence of an *N*-acetyl group, supported by a strong carbonyl absorption at 1630 cm^{-1} in the IR spectrum and two of acetyl methyl at δ 21.0 and 22.0 and two of carbonyl carbon signals at δ 171.2 and 170.8 in the ^{13}C NMR spectrum. Other paired signals were observed in the ^{13}C NMR spectrum as follows: δ 33.3 and 36.8 for *N*-methyl; δ 24.2, 51.4 and 23.3, 48.6 for the ethylene chain as well as for carbon atoms of the indole nucleus (see Experimental). Therefore, we formulated the alkaloid as a pair of rotamers of *N*-methyl-*N*-acetyltryptamine (MAT) (**2a** and **2b**) [6].

The fundamental structure was substantiated by the fragmentation pattern observed in the mass spectrum. The $[M]^+$, m/z 216, confirmed by CI mass spectrometry, $[M+1]^+$, m/z 217, was fragmented to m/z 143 (base peak) by loss of *N*-methyl acetamide (C_3H_7NO) and the peak m/z 130, C_9H_8N , was obtained by loss of methine from m/z 143. The structures of the rotamers were determined by comparison of ^1H NMR spectra between deuteriochloroform and deuterobenzene as follows: the rotamer with the smaller of the paired signals is **2b** and the one with larger is **2a** (Table 1) [4]. The rotamers of MAT were detected as two peaks using 2% *n*-butanol in chloroform on HPLC at 0° . MAT would rotate about the carbon-nitrogen bond more rapidly than MFT on the HPLC, because rotamers of the former (**2a** and **2b**) were separated by HPLC at a lower temperature (0°) than rotamers of the latter (**1a** and **1b**) (25°) [5].

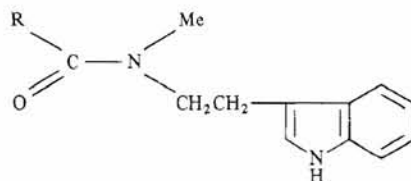
Subsequent elution of the chromatograph column with 30% methanol in chloroform gave an alkaloid which had the molecular formula $C_{12}H_{12}N_2$ by high resolution mass spectrometry. It was identified as 2-methyl-1,2,3,4-tetrahydro- β -carboline by comparison of its ^1H NMR (200 MHz) and mass spectra with those of the compound previously reported [7].

The first fraction eluted with methanol alone contained two compounds which were separated by prep. TLC. One of these, $C_{12}H_{16}N_2$, was determined to be DMT by high resolution mass spectrometry and ^1H NMR, and comparison of the spectra with those of the authentic material [8]. The other was identified as DMT oxide by downfield shifts of the methyl and ethylene protons on the nitrogen compared to those of DMT. A peak at $[M-16]^+$, m/z 188, was seen in the mass spectrum. DMT-oxide was reduced to DMT with ferrous sulphate in ammonia solution.

The third alkaloid was obtained from subsequent methanol eluates and identified as *N*-monomethyltryptamine (MMT) by ^1H NMR and mass spectra [8].

EXPERIMENTAL

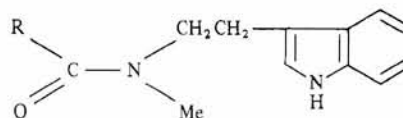
Bark of *Virola sebifera* Aublet (4.3 kg) was extracted with MeOH. After removal of solvent the residue was dissolved in 5% HOAc and filtered. The filtrate was basified with Na_2CO_3 and extracted with CHCl_3 to obtain a soln of crude alkaloids which was dried over Na_2SO_4 and concd under red. pres., and chromatographed on silica gel using gradient elution with CHCl_3 -MeOH. From the fraction eluted with CHCl_3 , an alkaloid was detected on silica gel TLC when sprayed with Dragendorff and Ehrlich reagents. It was purified by prep. TLC on silica gel using CH_2Cl_2 -MeOH- H_2O (4:3:1) to yield a non-crystalline substance, $C_{12}H_{14}N_2O$ (high resolution MS: observed 202.108; calc. 202.111); UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 287.5 (3.89), 277 (3.98), 271 (3.97), 237.5 (3.93); IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3250, 2920, 2845, 1645; ^1H NMR (200 MHz) *Z*-form (**1a**) (CDCl_3): δ 2.88 (s, *N*-CH₃), 3.05 (t, $J = 6.0$ Hz, CH₂), 3.70 (t, $J = 6.0$ Hz, CH₂), 7.10 (d, $J = 2.2$ Hz, H-2), 7.18 (td, $J = 8.0$ and 2.0 Hz, H-5 or H-6), 7.22 (td, $J = 8.0$ and 2.0 Hz, H-6 or H-5), 7.40 (dd, $J = 8.0$ and 2.0 Hz, H-4), 7.69 (dd, $J = 8.0$ and 2.0 Hz, H-7), 8.10 (s, CHO), 8.13 (br, NH); (C_6D_6): δ 1.97 (s, *N*-CH₃), 2.82 (t, $J = 6.0$ Hz, CH₂), 3.24 (t, $J = 6.0$ Hz, CH₂), 6.66 (d, $J = 2.0$ Hz, H-2), 7.16-7.30, 7.43-7.51, 7.66-7.74 (m, H-4, H-5, H-6, H-7, which could not be separately assigned to the individual isomers), 7.66 (s, CHO), 7.90 (b, NH); ^1H NMR (200 MHz) *E*-form (**1b**) (CDCl_3): δ 2.93 (s, *N*-CH₃), 3.04 (t, $J = 6.0$ Hz, CH₂), 3.54 (t, $J = 6.0$ Hz, CH₂), 6.99 (d, $J = 2.2$ Hz, H-2), 7.19 (td, $J = 8.0$ and 2.0 Hz, H-5 or H-6), 7.23 (td, $J = 8.0$ and 2.0 Hz, H-6 or H-5), 7.41 (dd, $J = 8.0$ and 2.0 Hz, H-4), 7.60 (dd, $J = 8.0$ and 2.0 Hz, H-7), 7.81 (s, CHO), 8.16 (b, NH); (C_6D_6): δ 2.52 (*N*-CH₃), 2.46 (t, J



Z-Form

1a R = H

2a R = Me



E-Form

1b R = H

2b R = Me

= 6.0 Hz, CH₂), 2.82 (t, *J* = 6.0 Hz, CH₂), 6.42 (d, *J* = 2.0 Hz, H-2), 7.16–7.30, 7.43–7.51 and 7.66–7.74 (m, H-4, H-5, H-6, H-7, see Z-form), 7.51 (s, CHO), 8.18 (br s, NH); ¹³C NMR (50 MHz) Z-form (1a) (CDCl₃): δ 22.8 (t, CH₂), 35.0 (q, N-CH₃), 44.9 (t, CH₂), 111.2 (d, C-7), 112.7 (s, C-3), 118.6 (d, C-4), 119.4 (d, C-5), 122.0 (d, C-6), 122.3 (d, C-2), 127.4 (s, C-3a), 136.7 (s, C-7a), 162.6 (d, CHO), ¹³C NMR (50 MHz) E-form (1b) (CDCl₃): δ 24.4 (t, CH₂), 29.7 (q, N-CH₃), 50.1 (t, CH₂), 111.5 (d, C-7), 111.6 (s, C-3), 118.2 (d, C-4), 119.6 (d, C-5), 122.1 (d, C-6), 122.4 (d, C-2), 126.9 (s, C-3a), 136.7 (s, C-7a), 162.9 (d, CHO); EIMS *m/z* (rel. int.): [M]⁺ 202 (9.8%), 143 (63.7), 130 (100); CIMS (isobutane) [M + 1]⁺ *m/z* 203.

The second compound eluted with 4% MeOH in CHCl₃ detected by Dragendorff and Ehrlich reagents on silica gel TLC, was purified by silica gel CC using gradient elution with cyclohexane and EtOAc followed by prep. TLC using CHCl₃-EtOAc (2:1) on NH₂ F₂₅₄S (Merck). It was a non-crystalline substance, C₁₃H₁₆N₂O (high resolution MS: observed 216.127; calc. 216.126); UV λ_{max}^{CHCl₃} nm (log ε): 2.83 (3.60), 277 (3.68), 270 (3.67), 236 (3.75); IR ν_{max}^{CHCl₃} cm⁻¹: 3500, 3000, 2930, 2850, 1630; ¹H NMR Z-form (2a) (CDCl₃): δ 2.10 (s, MeCO), 2.99 (s, N-CH₃), 3.04 (t, *J* = 7.0 Hz, CH₂), 3.61 (t, *J* = 7.0 Hz, CH₂), 7.01 (d, *J* = 2.2 Hz, H-2), 7.17 (td, *J* = 7.4 and 1.4 Hz, H-5 or H-6), 7.25 (td, *J* = 7.4 and 1.4 Hz, H-6 or H-5), 7.41 (dd, *J* = 7.4 and 1.4 Hz, H-4), 7.61 (dd, *J* = 7.4 and 1.4 Hz, H-7), 8.14 (b, NH); (C₆D₆): δ 1.66 (s, MeCO), 2.12 (s, N-CH₃), 2.98 (t, *J* = 7.0 Hz, CH₂), 3.60 (t, *J* = 7.0 Hz, CH₂), 6.40 (d, *J* = 2.2 Hz, H-2), 7.15–7.25, 7.44–7.52, 7.72–8.80 (m, H-4, H-5, H-6, H-7, which could not be separately assigned to the individual isomers), 7.66 (br, NH); ¹H NMR E-form (2b) (CDCl₃): δ 1.88 (s, MeCO), 2.92 (s, N-CH₃), 3.04 (t, *J* = 7.0 Hz, CH₂), 3.71 (t, *J* = 7.0 Hz, CH₂), 7.08 (d, *J* = 2.2 Hz, H-2), 7.15 (td, *J* = 7.4 and 1.4 Hz, H-5 or H-6), 7.23 (td, *J* = 7.4 and 1.4 Hz, H-6 or H-5), 7.39 (dd, *J* = 7.4 and 1.4 Hz, H-4), 7.69 (dd, *J* = 7.4 and 1.4 Hz, H-7), 8.06 (br, NH); (C₆D₆): δ 1.48 (s, MeCO), 2.72 (s, N-CH₃), 2.57 (t, *J* = 7.0 Hz, CH₂), 2.94 (t, *J* = 7.0 Hz, CH₂), 6.65 (d, *J* = 2.2 Hz, H-2), 7.15–7.25, 7.44–7.52, 7.72–8.80 (m, H-4, H-5, H-6, H-7, see Z-form), 7.32 (br, NH); ¹³C NMR Z-form (2a) (CDCl₃): δ 21.0 (q, CH₃CO), 24.2 (t, CH₂), 33.3 (q, N-CH₃), 51.4 (t, CH₂), 111.4 (d, C-7), 112.1 (s, C-3), 118.2 (d, C-4), 119.3 (d, C-5), 122.3 (d, C-2 and C-6), 128.0 (s, C-3a), 136.3 (s, C-7a), 171.2 (s, CH₃CO); ¹³C NMR E-form (2b) (CDCl₃): δ 22.0 (q, CH₃CO), 23.3 (t, CH₂), 36.8 (q, N-CH₃), 48.6 (t, CH₂), 111.2 (d, C-7), 113.2 (s, C-3), 118.7 (d, C-4), 119.6 (d, C-5), 122.0 (d, C-2 and C-6), 127.5 (s, C-3a), 136.3 (s, C-7a), 170.8 (s, CH₃CO); EIMS *m/z* (rel. int.): [M]⁺ 216 (7.6), 143 (100), 130 (82.3), CIMS (isobutane) [M + 1]⁺ *m/z* 217.

The chromatographic fraction eluted with 30% MeOH in CHCl₃ to yield an alkaloid which was purified by prep. TLC on silica gel using CH₂Cl₂-MeOH-H₂O (4:3:1) was identified as 2-

methyl-1,2,3,4-tetrahydro-β-carboline by spectroscopic comparison with the lit. [7].

The first fraction eluted with MeOH gave two alkaloids detected on TLC which were separated and isolated by prep. TLC using the above solvent system. One was identified as DMT by comparison with an authentic sample submitted by M. Endo; the other was determined to be DMT *N*-oxide from its spectral data.

DMT *N*-oxide (5 mg) was reduced by suspension in conc. aq. NH₃ (1 ml), excess FeSO₄ added and the mixture refluxed for 30 min. DMT was extracted with Et₂O and identified by TLC comparison with an authentic sample.

The last fraction eluted with MeOH gave an alkaloid purified by prep. TLC which was confirmed to be MMT by spectral characterization.

HPLC was performed on an instrument fitted with an A detector (254 nm) on a μ-Porasil column (300 × 3.9 mm) using CHCl₃-*n*-hexane (19:1) with a flow rate of 1.5 ml/min at 25° for MFT and a Lichrosorb 100 column (150 × 4.0 mm) using CHCl₃-*n*-BuOH (49:1) with a flow rate of 1.0 ml/min at 0° for MAT.

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