Phytochemistry, Vol. 21, No. 9, pp. 2389–2392, 1982.
Printed in Great Britain.

0031-9422/82/092389-04$03.00/0

ALSTOLENINE, 19,20-DIHYDROPOLYNEURIDINE AND OTHER MINOR ALKALOIDS OF THE LEAVES OF *ALSTONIA VENENATA**

PRIYALAL MAJUMDER and ASHOKE BASU

Department of Chemistry, University College of Science, Calcutta 700009, India

(*Revised received* 8 *January* 1982)

Key Word Index—*Alstonia venenata*; Apocynaceae; alstolenine; 19, 20-dihydropolyneuridine; polyneuridine; deacetylakuammiline; raucaffrinoline; indole alkaloids.

Abstract—Structures of alstolenine and 19,20-dihydropolyneuridine, two new indole alkaloids of the leaves of *Alstonia venenata* have been established. In addition, deacetylakuammiline, polyneuridine and raucaffrinoline have been isolated for the first time from this plant.

INTRODUCTION

Twenty new indole alkaloids and some six indolic bases of previously known structures, besides trimethylgallamide and the biogenetically significant pyridine base, venoterpine, have been isolated from different parts of *Alstonia venenata* R.Br. [1–3]. Further investigation of the strong basic fractions of the petrol and $CHCl_3$ extracts of the leaves of this plant involving large-scale extraction, pH-gradient fractionation of the total basic material, followed by repeated CC and prep. TLC has resulted in the isolation of two more new bases, alstolenine and 19,20-dihydropolyneuridine, besides deacetylakuammiline, polyneuridine and raucaffrinoline in extremely low yields (0.00002–0.0002%). The present communication deals with the structure elucidation of the two new bases, besides characterization of the three known alkaloids.

RESULTS AND DISCUSSION

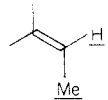
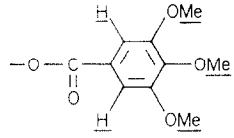
Alstolenine, $C_{31}H_{34}N_2O_7$ ($M^{+\cdot}$ 546), amorph., $[\alpha]_D$ −70.9° (EtOH), shows UV absorption typical of a 2,3-disubstituted indolenine chromophore [4]. This is also supported by characteristic absorption for $\diagdown$C=N– in its IR spectrum which, in addition, shows strong bands for both conjugated and unconjugated ester functions. That one of these is associated with a methyl ester and the other with a trimethylgalloyl moiety is evident from its ^{1}H NMR spectrum (Table 1) which also shows the presence of an ethylidene function and the four aromatic protons of the indolenine chromophore. The one-proton doublet at δ 4.65 is reminiscent of the C-3 hydrogen of an akuammiline type of alkaloid [5]. The marked hyperchromic effect at 268 nm in the UV spectrum of alstolenine also indicates the presence of a trimethylgalloyl moiety. The above spectral data are consistent with the structure **1a** for alstolenine. This is further supported by its characteristic mass spectral fragmentation [6] showing significant peaks at *m/z* 487 ($M^{+\cdot}$) − 59; loss of CO_2Me), 335 ($M^{+\cdot}$ − 211; loss of trimethylgalloyloxy), 321 ($M^{+\cdot}$ − 225; loss of trimethylgalloyloxymethylene), 276 ($M^{+\cdot}$ − 270; loss of CO_2Me and trimethylgalloyloxy), 249, 212, 195 and 168.

Chemical evidence in support of formula **1a** for alstolenine was provided by its alkaline hydrolysis furnishing trimethylgallic acid. Further, alstolenine on $LiAlH_4$ reduction afforded the indolinediol **1d** obtained by similar reduction of deacetylakuammiline (**1c**) [7] or akuammiline (**1b**). This establishes the stereochemistry of alstolenine to be identical with that of **1b**, while the illustrated stereochemistry of alstolenine (**1a**) at C-16 is based on the chemical shift of the protons of its CO_2Me group appearing at the normal position as in akuammiline (**1b**) itself [7]. An opposite stereochemistry at this centre (C-16) of alstolenine would have resulted in an extraordinary upfield shift of the methyl ester protons by the ring current effect of the indolenine moiety.

The second new alkaloid, $C_{21}H_{26}N_2O_3$ ($M^{+\cdot}$ 354), amorph. exhibits UV absorption characteristic of a 2,3-disubstituted indole chromophore [4]. Its IR spectrum shows bands for NH, OH and an unconjugated ester function. The ^{1}H NMR spectrum (Table 1) of the alkaloid establishes the presence of a CO_2Me group, a hydroxymethyl function attached to a quaternary carbon, an ethyl side-chain and an NH and four aromatic protons associated with the indole chromophore. The mass spectral fragmentation of the alkaloid showing significant peaks at *m/z* 353 ($M^{+\cdot}$ − 1), 337 ($M^{+\cdot}$ − 17; loss of OH), 336 ($M^{+\cdot}$ − 18; loss of H_2O), 325 ($M^{+\cdot}$ − 29; loss of CH_2Me), 323 ($M^{+\cdot}$ − 31; loss of CH_2OH), 295 ($M^{+\cdot}$ − 59; loss of CO_2Me), 224, 223, 169 and 168 is strikingly similar to that of a 19,20-dihydrosarpagine derivative [8]. Based on these spectral data the 19,20-dihydropolyneuridine struc-

*A preliminary account of this work was presented at the 'Annual Convention of Chemists', Kurukshetra, India in 1979; Abstracts of papers in *Org.* **83**, 32.

Table 1. 80 MHz ^{1}H NMR spectral data of alkaloids **1a** and **2a**

1a		**2a**	
δ_{ppm}(multiplicity)	Assignments	δ_{ppm}(multiplicity)	Assignments
3.72 (3H, *s*)	$-CO_2\underline{Me}$	0.82 (3H, *t*, J = 7 Hz)	$-CH_2\underline{Me}$
1.68 (3H, *d*, J = 7 Hz) and		3.34 (2H, AB*q*, J = 12 Hz)	$-C(-\underline{CH_2}-OH)-$
5.52 (1H, *q*, J = 7 Hz)		3.78 (3H, *s*)	$-CO_2\underline{Me}$
3.89 (9H, *s*) and 6.97 (2H, *s*)		4.23 (1H, *br* signal)	$Ar-NH-\underline{C}(-\underline{C}\underline{H}-N-)-$
4.65 (1H, *d*, J = 4.5 Hz)	C-3 $\underline{H}$	7.03–7.49 (4H, *m*)	Ar-$\underline{H}$
6.9–7.78 (4H, *m*)	Ar-$\underline{H}$	7.73 (1H, *br s*, disappeared on deuterium exchange	$-N-\underline{H}$

ture **2a** was assigned to the alkaloid, the stereochemistry at C-16 being settled from the normal chemical shift (δ3.78) of its methyl ester protons. In the alternative 19,20-dihydroakuammidine formulation **2d** these protons would have suffered a large upfield shift as in the case of akuammidine (**2c**) [9–11]. The stereochemistry at C-20 of the alkaloid, however, still remains undefined.

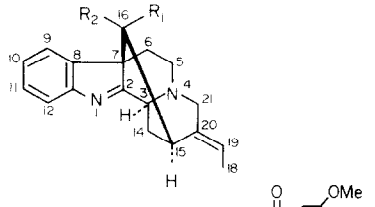
One of the remaining three alkaloids was identified as deacetylakuammiline (**1c**) [7] by direct comparison with an authentic sample prepared by mild acid hydrolysis of akuammiline (**1b**) [7]. The latter was also found to be identical with the acetyl derivative of the alkaloid. The other two alkaloids are believed to be polyneuridine (**2b**) [11, 12] and raucaffrinoline [13] on the basis of their physical constants and spectral data, although direct comparison with authentic samples was not possible.

The isolation of these alkaloids from the leaves of *A. venenata* is of biogenetic significance, in that it reveals the occurrence in this plant of indole alkaloids of three more structural types, viz. akuammiline, sarpagine and modified ajmaline, in addition to the yohimbine, aspidofractinine and vincadifformine types of alkaloids isolated earlier.

EXPERIMENTAL

Mps are uncorrected. Brockmann Al_2O_3 and Si gel (60–80 mesh) were used for CC and Si gel G for TLC performed at 25–30°. UV spectra were measured in 95% aldehyde-free EtOH and IR spectra were run in KBr discs. ^{1}H NMR spectra were recorded at 80 MHz in $CDCl_3$ using TMS as the int. standard. Mass spectra were run by direct insertion at 70 eV, and the figures in the first bracket attached to the *m/z* values represent the relative intensities of the peaks. All analytical samples were routinely dried over P_2O_5 at 80° for 24 hr *in vacuo* and were tested for purity by TLC and MS. Dry Na_2SO_4 was used for drying and petrol used had bp 60–80°.

Isolation of alstolenine (**1a**), *19,20-dihydropolyneuridine* (**2a**), *deacetylakuammiline* (**1c**), *polyneuridine* (**2b**) *and raucaffrinoline.* Air-dried powdered leaves (2.5 kg in a batch; total amount extracted 20 kg) of *A. venenata* were successively extracted with petrol and $CHCl_3$ in a Soxhlet. The petrol and $CHCl_3$ extracts of each batch were concentrated, combined and mixed with 5% aq. citric acid (2 l.) and filtered to remove the nonbasic material. The filtrate was exhaustively extracted successively with C_6H_6 and $CHCl_3$ to remove weak bases. The aq. citrate soln containing the strongly basic compound was basified with NH_4OH in the cold and the liberated bases were extracted with $CHCl_3$, washed, dried, concentrated and chromatographed over Al_2O_3. The chromatogram was exhaustively washed with C_6H_6–$CHCl_3$ (4:1) which removed the previously known alkaloids. It was then eluted with $CHCl_3$. The early fractions of the $CHCl_3$ eluate on evapn gave a gummy mass (0.05 g) of alkaloidal mixture. The remaining seven batches of the plant materials were processed exactly as above to obtain alkaloidal fractions. The combined mixture of alkaloids, thus obtained was repeatedly chromatographed over Si gel. The 5% methanolic $CHCl_3$ eluate gave a mixture of six alkaloids of very close polarity. Repeated TLC of this mixture in EtOAc–$CHCl_3$–MeOH (5:1:1) (×3), followed by further

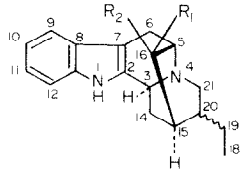


1a $R_1 = CO_2Me$, $R_2 = CH_2-O-C(=O)$-(3,4,5-trimethoxyphenyl)

1b $R_1 = CO_2Me$, $R_2 = CH_2OAc$

1c $R_1 = CO_2Me$, $R_2 = CH_2OH$

1d 1,2-dihydro, $R_1 = R_2 = CH_2OH$



2a $R_1 = CO_2Me$, $R_2 = CH_2OH$

2b 19,20-dehydro, $R_1 = CO_2Me$, $R_2 = CH_2OH$

2c 19,20-dehydro, $R_1 = CH_2OH$, $R_2 = CO_2Me$

2d $R_1 = CH_2OH$, $R_2 = CO_2Me$

purification by CC afforded pure **1a** (yield, 0.0002%), alstovenine (yield, 0.0001%), **2a** (yield, 0.0001%), **1c** (yield, 0.0001%), **2b** (yield, 0.00004%) and raucaffrinoline (yield, 0.00002%).

Alstolenine (**1a**), amorph., $[\alpha]_D - 70.9°$ (EtOH); R_f 0.40 in EtOAc–$CHCl_3$–MeOH (6:1:1); produces pink colour with ceric $(NH_4)_2SO_4$. (Calc. for $C_{31}H_{34}N_2O_7$: C, 68.13; H, 6.23; N, 5.13. Found: C, 68.24; H, 6.29; N, 5.18%.) λ_{max} 216 and 268–270 nm (log ϵ 4.59 and 4.15); subtraction UV from trimethylgallic acid, λ_{max} 216 and 268 nm (log ϵ 4.17 and 3.62); ν_{max} 1735 (CO_2Me), 1715 (Ar–C(=O)–O), 1595 (>C=N–), 755, 730 (1,2-disubstituted C_6H_6) cm^{-1}; *m/z* 546 ($M^{+\cdot}$ 84), 531 (17), 487 (14), 335 (22), 321 (100), 276 (7.5), 250 (4.5), 249 (18), 248 (8.5), 247 (12), 232 (12), 212 (8.5), 195 (62.5), 179 (16), 170 (4.5), 168 (16); *m*/z* 434.3 and 188.7.

19,20-Dihydropolyneuridine (**2a**), amorph., R_f 0.35 in EtOAc–$CHCl_3$–MeOH (6:1:1). (Calc. for $C_{21}H_{26}N_2O_3$: C, 71.18; H, 7.35; N, 7.91. Found: C, 71.25; H, 7.40; N, 7.89%.) λ_{max} 226, 282 and 290 nm (log ϵ 4.44, 3.81 and 3.74); λ_{max} 3520 (OH), 3380 (NH), 1725 (CO_2Me), 745, 725 (1,2-disubstituted C_6H_6) cm^{-1}; *m/z* 354 ($M^{+\cdot}$, 70), 353 (100), 337 (5.3), 336 (12), 325 (4), 323 (5), 295 (8), 253 (5), 251 (5), 250 (5.5), 249 (18), 237 (5), 236 (7.5), 235 (7), 234 (6.5), 226 (5), 225 (15), 224 (20), 223 (8), 222 (5), 221 (9), 220 (5), 219 (5), 211 (7.5), 210 (8), 209 (9), 208 (5.5), 207 (5.5), 198 (8.5), 197 (10), 196 (5), 195 (8), 194 (6.5), 185 (11), 184 (20), 183 (15), 182 (10), 181 (6.5), 180 (9), 171 (13.5), 170 (38), 169 (60), 168 (25), 167 (25), 166 (6), 130 (14), 129 (16), 123 (12) and 121 (8).

Deacetylakuammiline (**1c**), amorph., $[\alpha]_D + 14°$ (EtOH); R_f 0.3 in EtOAc–$CHCl_3$–MeOH (4:1:1); produces pink colour with ceric $(NH_4)_2SO_4$; λ_{max} 216, 220 and 266–268 nm (log ϵ 4.11, 4.11 and 3.58); ν_{max} 3370 (OH), 1735 (CO_2Me), 1600 (>C=N–), 760 and 730 (1,2-disubstituted C_6H_6) cm^{-1}; δ_{ppm} 1.62 (3H, *d*, *J* = 7 Hz; MeCH=C) 3.78 (3H, *s*; CO_2Me), 4.54 (1H, *d*, *J* = 4.5 Hz; Ar–N=C–CH–N–), 5.42 (1H, *q*, *J* = 7 Hz; MeCH=C) and 7.01–7.62 (4H, *m*; Ar–H); *m/z* 352 ($M^{+\cdot}$, 70), 335 (4.5), 321 (100), 293 (20), 250 (4.5), 249 (15), 248 (10), 247 (13), 232 (18), 170 (5.5), 168 (12), 167 (13.5) *and* 121 (8).

Polyneuridine (**2b**), mp 242°, $[\alpha]_D - 69°$ (Py); R_f 0.33 in EtOAc–$CHCl_3$–MeOH (6:1:1); λ_{max} 226, 282 and 290 nm (log ϵ 4.43, 3.79 and 3.73); ν_{max} 3510 (OH), 3370 (NH), 1725 (CO_2Me), 745 and 725 (1,2-disubstituted C_6H_6) cm^{-1}; *m/z* 352 ($M^{+\cdot}$, 100), 351 (60), 337 (5), 335 (25), 334 (25), 321 (70), 293 (45), 251 (5), 250 (7), 249 (20), 248 (15), 247 (20), 236 (8), 235 (6), 234 (5), 233 (18), 232 (16), 224 (21), 223 (10), 222 (5), 221 (10), 209 (9), 208 (5.5), 207 (5.5), 206 (30), 205 (20), 193 (28), 192 (25), 184 (30), 183 (20), 182 (12), 181 (10), 180 (11), 171 (17), 170 (47), 169 (70), 168 (30), 165 (30), 164 (15), 130 (15), 129 (20) and 121 (10).

Raucaffrinoline, amorph. R_f 0.30 in EtOAc–$CHCl_3$–MeOH (6:1:1); produces pink colour with ceric $(NH_4)_2SO_4$; λ_{max} 215 and 263 nm (log ϵ 4.11 and 3.57); ν_{max} 3500 (OH), 1235 and 1725 (OAc), 1590 (>C=N–), 755 and 725 (1,2-disubstituted C_6H_6) cm^{-1}; *m/z* 352 (M^+; 96), 351 (13.5), 337 (4), 335 (5.7), 322 (27), 321 (100), 306 (4), 293 (15.5), 292 (7.5), 280 (9.5), 279 (50), 264 (5), 263 (7.5), 262 (9.5), 261 (9.5), 250 (5), 249 (11.5), 248 (7.5), 247 (8), 238 (7), 234 (7), 233 (10), 232 (12), 223 (5), 197 (4), 194 (9.5), 193 (6), 192 (6), 191 (4), 182 (6.5), 181 (7), 180 (7), 169 (10), 168 (22), 154 (9.5), 152 (4), 150 (20), 130 (7), 129 (10.5), 121 (8), and 115 (9).

Alkaline hydrolyis of alstolenine (**1a**). A soln of **1a** (0.012 g) in 5% methanolic KOH (3 ml) was refluxed for 1 hr. MeOH was removed under red. pres. and the residue was diluted with H_2O, acidified with HCl in the cold, extracted with Et_2O, washed and dried. The Et_2O extract on evapn gave trimethylgallic acid (0.004 g), crystallized from MeOH in fine needles, mp 168°.

Reduction of alstolenine (**1a**) *and akuammiline* (**1b**). A soln of **1a** (0.007 g) in 5 ml THF was added dropwise to a slurry of $LiAlH_4$ (0.015 g) in THF (10 ml) at 0° under anhydrous condition with continuous stirring. The mixture was then refluxed for 5 hr. The soln was cooled, excess reagent was destroyed with EtOAc and the product was treated with ice-cold H_2O. It was filtered and the residue was repeatedly washed with boiling $CHCl_3$. The filtrate was evaporated and extracted with $CHCl_3$, dried and concentrated. TLC of the product produced mainly a single Dragendorff's staining spot having R_f 0.15 in EtOAc–EtOH (3:1). It was identified by co-TLC with the major product (**1d**) of $LiAlH_4$ reduction of **1b** (0.01 g) carried out under identical conditon.

Acetylation of deacetylakuammiline (**1c**). **1c** was acetylated with Ac_2O–py at room temp. The product after usual work-up was chromatographed over Al_2O_3. The petrol–EtOAc (6:1) eluate on evapn gave akuammiline (**1b**) (0.007 g), crystallized from MeOH, mp 157–160°.

Mild acid-hydrolysis of akuammiline (**1b**). **1b** (0.008 g) was treated with 3 N aq. HCl (1 ml) and the soln was kept at room temp. for 24 hr. It was then basified in the cold with NH_4OH, extracted with Et_2O, dried and the solvent removed. The residue was chromatographed over Al_2O_3. The column was washed first with petrol–EtOAc (6:1) to (1:1) to give **1c** (0.005 g).

Acknowledgements—The authors thank Dr. B. C. Das, C.N.R.S., Gif-sur-Yvette, France, Dr. G. F. Smith, University of Manchester, and Dr. D. A. Evans, University of Southampton, for the mass spectra and Professor J. Levy, Faculty of Pharmacy, University of Reims, for an authentic sample of akuammiline. The work was supported by C.S.I.R., India.

REFERENCES

1. Majumder, P. L., Joardar, S., Dinda, B. N., Bandyopadhyay, D., Joardar, S. and Basu, A. (1981) *Tetrahedron* **37**, 1243.
2. Chatterjee, A., Mukhopadhyay, S. and Shoolery, J. N. (1978) *Indian J. Chem.* **16B**, 67.
3. Mitscher, L. A., Ray, A. B. and Chatterjee, A. (1971) *Experientia* **27**, 16.
4. Neuss, N. (1962) *Physical Data of Indole and Dihydroindole Alkaloids*. Lilly, Indianapolis.
5. Dugan, J. J., Hesse, M., Renner, U. and Schmid, H. (1969) *Helv. Chim. Acta* **52**, 701.
6. Oliver, L., Levy, J., Lemen, J., Janot, M. M., Budzikiewicz, H. and Djerassi, C. (1964) *Ann. Pharm. Fr.* **22**, 35.
7. Oliver, L., Levy, J., LeMen, J., Janot, M. M., Budzikiewicz, H. and Djerassi, C. (1965) *Bull. Soc. Chim. Fr.* 868.

8. Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. 1, pp. 77–88. Holden-Day, San Francisco.
9. Chatterjee, A., Ghosal, C. R. and Adityachaudhury, N. (1962) *J. Sci. Ind. Res.* **21B**, 147.
10. Silvers, S. and Tulinsky, A. (1962) *Tetrahedron Letters* 339.
11. Antonaccio, D., Pereira, N. A., Gilbert, B., Vorbrueggen, H., Budzikiewicz, H., Wilson, J. M., Durham, L. J. and Djerassi, C. (1962) *J. Am. Chem. Soc.* **84**, 2161.
12. Janot, M. M., LeMen, J., Gosset, J. and Levy, J. (1962). *Bull. Soc. Chim. Fr.* 1079.
13. Khan, M. A. and Siddique, S. (1972) *Experientia* **28**, 127.