INDOLE ALKALOIDS FROM *VINCA SARDOA*, A NEW SPECIES OF *VINCA*

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Abstract-Four natural *N*-methylindolines have been isolated from the roots of *Vinca sardoa* (Stearn) Pignatti. They are *ent*-*N*(1)-methyl-14,15-didehydroaspidospermidine (**7**), *N*(1)-methyl-14,15-didehydroaspidofractinine (**10**), *N*(1)-methylaspidofractinine (**11**) and *N*(1)- methyl-14,15-didehydrotuboxenine (**13**).

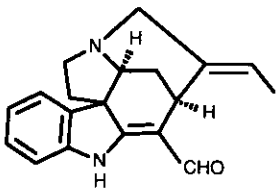
We have undertaken an investigation of alkaloid constituents of the roots of *Vinca sardoa* (Stearn) Pignatti,[1] a new species of *Vinca* native of Sardinia, in order to search for new compounds. The major indole alkaloids were isolated from the methanolic extract of the roots by means of repeated column, preparative chromatography and crystallization. We report here the identification of these compounds giving a full account of the structural analysis of the new bases.

Six of these were known and identified by means of their physical and spectroscopic data as norfluorocurarine (**1**),[2,3] akuammigine (**2**),[4] carapanaubine (**3**),[5] majdine (**4**),[6] isomajdine (**5**)[6] and rauvoxinine (**6**).[7] The other four compounds were all new natural *N*-methylindolines possessing a plumeran-type skeleton.

Alkaloid (**7**), *ent*-*N*(1)-methyl-14,15-didehydroaspidospermidine, showed uv maxima at 208,257 and 300 nm characteristic for an *N*-alkylindoline chromophore. Its mass spectrum displayed a $[M]^{+\cdot}$ at *m/z* 294 and was reminiscent to that of aspidospermidine (**8**)[8] except that peaks due to the indole moiety and aliphatic portion in **7** were 14 mass units above and 2 mass units less, respectively. In the ^1H nmr spectrum of **7** only a few signals could be attributed, namely a singlet at δ 2.74 due to *N*-Me group and a multiplet centered at δ 5.55 for two olefinic protons. The signals of four consecutive aromatic protons were also present in the downfield region of the spectrum. These data led to structure (**7**) for this alkaloid and this proposal was supported by the ^{13}C nmr spectrum which showed the correct number of signals with appropriate multiplicity (see Table).

An alkaloid of similar structure has been reported for a compound isolated from the reductive hydrolysis of the dimeric alkaloid (+)-pycnanthinin.[9] However, owing to the non-availability of an authentic sample, or physical or spectral data, the identity of **7** with the reported alkaloid could not be confirmed. Unequivocal proof of the structure and absolute configuration of **7** was performed comparing it with the corresponding compound prepared from (-)-tabersonine (**9**) through acid-catalyzed hydrolysis to decarbomethoxytabersonine[10] followed by reductive methylation. Not only the spectral data of both natural and synthetic **7** were coincident, but also their cd curves were superimposable, clearly indicating that **7** belonged to the same steric series as (-)-**9** (α series), enantiomeric to that of natural (+)-aspidospermidine (**8**).[11]

Two of the other alkaloids have been found to be *N*(1)-methyl-14,15-didehydroaspidofractinine (**10**) and the closely related *N*(1)-methylaspidofractinine (**11**). Both compounds displayed the same alkylindoline chromophore (208, 257 and 300 nm) in the uv spectrum and similar mass spectra. **10** showed a $[M]^{+\cdot}$ at *m/z* 292 and fragments at *m/z* 264 $[M\text{-}C_2H_4]^{+\cdot}$,172 and 158 (from indole ring), accompanied by small peaks at *m/z* 122 and 107 (from aliphatic piperidine moiety).[12] The mass spectrum of **11** showed a molecular peak at *m/z* 294 and piperidine fragments (*m/z* 124 and 109), which appeared 2 mass units above the corresponding ions of **10**. The presence of common intense peaks at *m/z* 172 and 158, featuring the indole portion of the molecule, suggested that the



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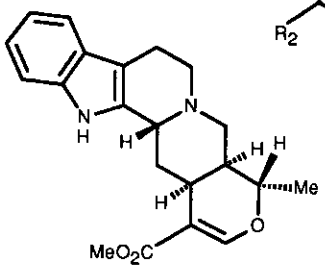

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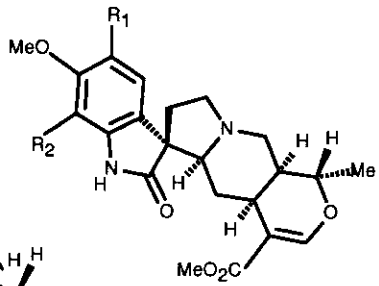
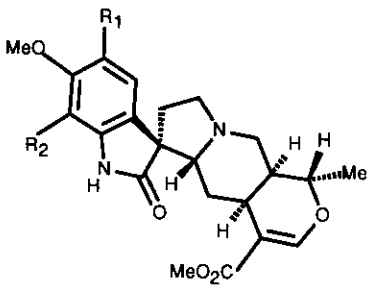


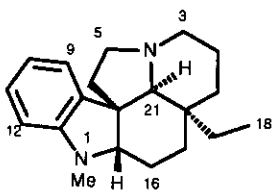
3 R_1=H; R_2=OMe

4 R_1=OMe; R_2=H



5 R_1=H; R_2=OMe

6 R_1=OMe; R_2=H



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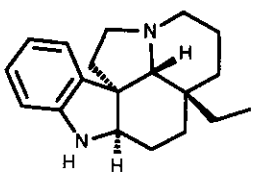

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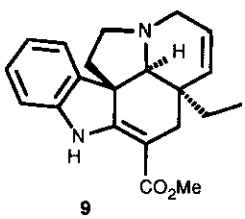

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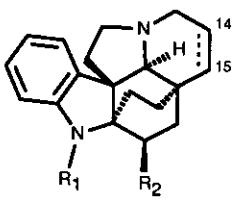
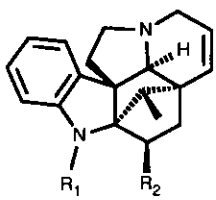


10 R_1=Me ; R_2=H ; Δ^{14}

11 R_1=Me ; R_2=H

12 R_1=H ; R_2=CO_2Me ; Δ^{14}



13 R_1=Me ; R_2=H

14 R_1=Me ; R_2=CO_2Me

15 R_1=H ; R_2= CO_2Me

supplementary 2 mass units were present on the aliphatic part of **11**.

The ^{1}H nmr specrum of **10** which showed, at variance to **11**, a multiplet for two olefinic protons at δ 5.3-5.8 easily indicated that the two compounds differed only for a double bond. Furthermore, reduction of **10** with hydrogen on Pd/C catalyst in methanol produced **11** in almost quantitative yield. The ^{1}H nmr spectra of **10** and **11** could not be confidently analyzed because of inadequate dispersion of the signals and only one significant structural informations could be gained. In addition to a *N*-Me singlet at δ 2.58 and δ 2.60, respectively, the absence of signals attributable to ethyl group strongly suggested an aspidofractinine-like structure for both compounds. The same conclusion could be deduced from the ^{13}C nmr spectrum of **10** (Table 1) which exhibited resonances very closed to those of venalstonine (**12**)[13] with the exception of those of C-16 (δ 22.6) and C-17 (δ 24.5) *vs* δ 43.4 and δ 29.6, respectively, in **12** in which the same carbons were deshielded by a C-16 carbomethoxy group.

Finally, absolute configuration of **10** and **11** was determined by the sign of the $\pi \rightarrow \pi^*$ transition in the 250 nm region of their cd spectra[14] (see Experimental).

Once the structure of indoline (**10**) had been determined, a structure for the last base (**13**) could be proposed mainly upon spectral comparison. **13** analyzed for $C_{20}H_{24}N_2$ by high-resolution mass spectrometry and showed uv absorption for an alkylindoline chromophore. The ^{1}H nmr features of **13** also compared favourably to those of **10** showing a dd at δ 6.20 (H-15), a ddd at δ 5.72 (H-14), a singlet at δ 2.85 (N-Me) and very similar aromatic signals and upfield region. However, the presence of a sharp doublet (J=7.2 Hz) at δ 0.98 (Me-18) led easily to propose the structure of *N*(1)-methyl-14,15-didehydrotuboxenine (**13**). This feature was confirmed comparing ^{13}C resonances of **13** (Table 1) with analogous ones in *N*(1)-methylvindolinine (**14**).[13] Minor differences in the C-16 and C-17 chemical shifts were readily attributable to the supplementary carbomethoxy group at C-16 in **14**.

A literature search revealed that a non-separable mixture of **13** and its 19-epimer had been obtained by Hesse[15] from the reductive cleavage (Zn/HCl) of the dimeric alkaloid (+)-pycnanthine. Although ^{1}H nmr and Eims data reported by Hesse were similar to those of **13**, no argument was given to substantiate its steric series.

To complete the structural work, it remain to establish the absolute configuration of **13**. Toward this end, the cd spectrum of **13** [$\Delta\epsilon_{238}$ −0.5, $\Delta\epsilon_{261}$+2.8, $\Delta\epsilon_{285}$+0.1, $\Delta\epsilon_{315}$+0.4] was compared to that of natural vindolinine (**15**) [$\Delta\epsilon_{226}$+0.6, $\Delta\epsilon_{246}$+2.3, $\Delta\epsilon_{271}$+0.4, $\Delta\epsilon_{302}$+1.2] whose chirality was known through X-ray analysis[16] and correlation.[17] The close similarity between the two spectra strongly suggested that both compounds **13** and **15** belonged to the same steric series (α series). Accordingly, the structure shown for **13** also represents the absolute configuration.

Table. ^{13}C nmr of alkaloids (**7**), (**10**) and (**13**).

	7	**10**	**13**		**7**	**10**	**13**
C-2	66.5	68.5	83.4	C-13	150.3	151.7	151.4
C-3	53.4	49.5	58.1	C-14	122.6	126.1	127.6
C-5	53.4	50.6	50.4	C-15	134.5	133.9	131.9
C-6	39.9	36.1	35.1	C-16	20.8	22.6	26.7
C-7	52.0	55.1	58.8	C-17	27.8	24.5	19.5
C-8	136.7	139.3	137.1	C-18	7.8	28.9	8.9
C-9	121.5	120.9	123.0	C-19	29.0	30.1	43.7
C-10	117.1	117.9	118.3	C-20	38.8	34.3	48.4
C-11	127.4	126.9	127.3	C-21	72.8	67.2	76.9
C-12	106.4	107.6	106.0	N-Me	31.6	29.5	30.7

EXPERIMENTAL

Uv spectra (MeOH solutions) were recorded on a Perkin-Elmer 554 Uv-vis spectrophotometer, ir spectra (NaCl disks) were taken on a Perkin-Elmer 681 spectrophotometer, ^{1}H nmr (100 MHz) and ^{13}C nmr (25.2 MHz) spectra

on a Varian XL-100 spectrometer using tetramethylsilane as internal standard. $[\alpha]_D$ values were obtained on a Perkin-Elmer model 141 polarimeter and cd measurements were carried out on a Jobin-Yvon Dichrograph III. Mass spectra were registered on Varian 112 and CH-7 spectrometers. Mps are uncorrected and were obtained on a capillary apparatus; tlc was run on silica gel 60 F_{254} Merck in the following solvent systems; A: $CHCl_3$-MeOH-H_2O, (75:24:1); B: CH_2Cl_2-MeOH-NH_4OH{d 1.8} (90:10:1).

Extraction: The dried and coarsely ground roots (3.3 Kg) of *Vinca sardoa* were stirred in MeOH (10 l) at room temperature for 5 h and the suspension drained. Extraction was repeated (5 x 5 l) until a Mayer's reagent test was negative and the combined methanolic extracts were concentrated under reduced pressure at room temperature to a volume of approximately 0.5 l. This residue was diluted with an aqueous solution of 5% citric acid (2 l), stirred for 2 h, and the resulting suspension was filtered through a celite pad. The clear filtrate was basified to pH 8 with 25% NH_4OH and extracted with CH_2Cl_2 (6 x 0.5 l). The CH_2Cl_2 solution was washed with H_2O, dried (Na_2SO_4) and evaporated *in vacuo* to give 31 g (0.9%) of crude alkaloid mixture.

Separation of alkaloids: Alkaloid mixture (31 g) was chromatographed on 600 g of neutral Al_2O_3 (grade III). Gradient elution was effected with C_6H_6, C_6H_6-Et_2O, Et_2O and Et_2O-MeOH mixtures yielding four fractions: A (eluted with C_6H_6; 7.7 g), B (eluted with gradient of Et_2O in C_6H_6 to pure Et_2O; 6.8 g), C (eluted with Et_2O-MeOH 7:1; 8.1 g) and D (eluted with Et_2O-MeOH 4:1; 7.5 g) . The first one, which exhibited four major spots, was rechromatographed over silica gel (500 g) and eluted with EtOAc to afford sequentially crude **10** (0.8 g), **7** (0.45 g), **11** (0.28 g) and **13** (1.7 g). Fraction B was chromatographed on a silica gel column eluting with mixtures of CH_2Cl_2 and MeOH. The fractions eluted with a 99:1 mixture gave crude akuammigine (**2**) (1.95 g) whereas elution with 97:3 mixture gave crude norfluorocurarine (**1**) (0.85 g). Fraction C was subjected to silica gel column and eluted with EtOAc giving sequentially crude isomajdine (**5**) (0.4 g) and majdine (**4**) (3.2 g).

The most polar fraction D was chromatographed on a silica gel column with stepwise elution with CH_2Cl_2-MeOH as eluant (increasing methanol content). Elution with CH_2Cl_2 gave crude carapanaubine (**3**) (3.03 g), whereas elution with CH_2Cl_2-MeOH (96:4) afforded crude rauvoxinine (**6**) (1.6 g).

The yields, physical properties and spectral data of purified isolated compounds are as follows.

Norfluorocurarine (**1**). Purified by repeated crystallization from EtOAc (0.2 g), mp 184-185°C; (lit.[2] mp 182-183°C).

Akuammigine (**2**). Colourless crystals from EtOAc-hexane (0.42 g), mp 122-124°C; (lit.[4] mp 125°C).

Carapanaubine (**3**). Colourless crystals from MeOH (0.85 g), mp 220-223°C; $[\alpha]_D^{25}$ −74° (c 0.5, MeOH); (lit.[5] mp 223°C).

Majdine (**4**). Isolated as its hydrobromide; colourless crystals from acetone (0.83 g); mp 205-208°C.

Isomajdine (**5**). Colourless crystals from CH_2Cl_2 (0.15 g); mp 208°C; $[\alpha]_D^{25}$ −57° (c 0.5, MeOH); (lit.[6] mp 208-210°C).

Rauvoxinine (**6**). Colourless crystals from MeOH (0.27 g); mp 200-202°C; $[\alpha]_D^{25}$ +58° (c 0.5, MeOH); (lit.[7] mp 203°C).

ent-N(1)-Methyl-14,15-didehydroaspidospermidine (**7**). Colourless crystals from petroleum ether (90 mg); mp 118-120°C; tlc, R_f 0.42 (eluent A); uv λ_{max}nm (log ϵ): 208 (4.17), 257 (3.76), 300 (3.32); ir ν_{max} cm^{-1}: 1605; Eims *m/z* (rel. int.): 294 $[M]^{+\cdot}$ (69), 266 (5), 265 $[M\text{-}C_2H_5]^+$ (11), 158 (38), 150 (8), 144 (25), 135 (100), 122 (21), 121 (33), 107 (23); ^1H nmr ($CDCl_3$): δ 7.06 (1H, t, J=8Hz, H-11), 6.98 (1H, d, J=8Hz, H-9), 6.38 (1H, m, H-12), 5.55 (2H, m, H-14 + H-15), 3.30 (1H, s, H-21), 3.29 (1H, m, H-2), 2.74 (3H, s, N-Me), 0.72 (3H, t, J=7.2Hz, H-18); $[\alpha]_D^{25}$ +12° (c 0.63, MeOH); $\Delta\epsilon_{248}$−3.1, $\Delta\epsilon_{265}$+4.3, $\Delta\epsilon_{292}$−0.11, $\Delta\epsilon_{322}$+2.45; Found: C, 81.63; H, 8.95; N, 9.58. $C_{20}H_{26}N_2$ requires: C, 81.58; H, 8.90; N, 9.51.

N(1)-Methyl-14,15-didehydroaspidofractinine (**10**). The amorphous colourless base was purified as its hydrobromide from EtOH (210 mg); mp 285-295°C; tlc, R_f 0.57 (eluent A); uv λ_{max}nm (log ϵ): 208 (4.58), 257 (4.08), 300 (3.64); ir, ν_{max} cm^{-1}: 1608; Eims *m/z* (rel. int.): 292 $[M]^+$(59), 264 (19), 172 (100), 156 (14), 144 (10),

122 (10), 107 (33); ^{1}H nmr ($CDCl_3$): δ 7.10 (1H, dd, J=8, 2.5Hz, H-9), 7.03 (1H, dt, J=8, 2.5Hz, H-11), 6.66 (1H, dt, J=8, 2.5Hz, H-10), 6.40 (1H, dd, J=8, 2.5Hz, H-12), 5.8-5.3 (2H, m, H-14 + H-15), 3.45 (2H, m, H-3), 2.58 (3H, s, N-Me); $\Delta\epsilon_{232}$–1.2, $\Delta\epsilon_{257}$+5.6, $\Delta\epsilon_{298}$–0.4; Found: C, 64.38; H, 6.78; N, 7.44. $C_{20}H_{25}BrN_2$ requires: C, 64.34; H, 6.74; N, 7.50.

N(1)-Methylaspidofractinine (**11**). Purified by tlc (eluent B) (30 mg); amorphous colourless solid; tlc, R_f 0.54 (eluent B); uv λ_{max}nm (log ε): 208 (4.13), 257 (3.70), 300 (3.15); ir ν_{max} cm^{-1}: 1606; Eims *m/z* (rel. int.): 294 $[M]^{+\cdot}$ (95), 266 (75), 172 (33), 158 (12), 124 (73), 109 (100); ^{1}H nmr ($CDCl_3$): δ 7.26 (1H, dd, J=8, 1.2Hz, H-9), 7.10 (1H, dt, J=8, 1.2Hz, H-11), 6.74 (1H, dt, J=8, 1.2Hz, H-10), 6.45 (1H, d, J=8Hz, H-12), 3.06 (1H, s, H-21), 2.60 (3H, s, N-Me); $\Delta\epsilon_{230}$–1.0, $\Delta\epsilon_{255}$+4.7, $\Delta\epsilon_{299}$–0.1; Found: C, 81.66; H, 8.88; N, 9.53. $C_{20}H_{26}N_2$ requires: C, 81.58; H, 8.90; N, 9.51.

N(1)-Methyl-14,15-didehydrotuboxenine (**13**). The amorphous colourless base was purified as its hydrobromide from EtOH (580 mg), mp 246-250°C; tlc, R_f 0.10 (eluent A); uv λ_{max}nm (log ε): 209 (4.35), 258 (3.94), 3.07 (3.43); ir ν_{max}cm^{-1}: 1605; Eims *m/z* (rel. int.): 292 $[M]^{+\cdot}$ (98), 263 (6), 186 (100), 185 (53), 171 (51), 170 (98), 158 (65), 134 (55); ^{1}H nmr ($CDCl_3$): δ 7.18 (1H, dd, J=7.5, 1.2Hz, H-9), 7.08 (1H, dt, J=7.5, 1.2Hz, H-11), 6.72 (1H, dt, J=7.5, 1.2Hz, H-10), 6.42 (1H, dd, J=7.5, 1.2Hz, H-12), 6.20 (1H, dd, J=10.2, 2.3Hz, H-15), 5.72 (1H, ddd, J=10.2, 4.0, 2.0Hz, H-14), 3.95 (1H, dd, J=17.5, 4.0Hz, H-3), 2.85 (3H, s, N-Me), 0.98 (1H, d, J=7.2Hz, H-18); $[\alpha]_D^{25}$+65° (c 0.5, $CHCl_3$); $\Delta\epsilon_{238}$–0.5, $\Delta\epsilon_{261}$+2.8, $\Delta\epsilon_{285}$+0.1, $\Delta\epsilon_{315}$+0.4; Found: C, 64.43; H, 6.68; N, 7.45. $C_{20}H_{25}BrN_2$ requires : C, 64.34; H, 6.74; N, 7.50.

Decarbomethoxytabersonine from tabersonine (**9**). This compound was prepared in 94% yield by the method of Le Men,[10] by heating **9** at 110°C with 4N hydrochloric acid.

Reductive methylation of decarbomethoxytabersonine. A mixture of MeOH (5 ml), acetic acid (0.3 ml), sodium acetate trihydrate (210 mg) and decarbomethoxytabersonine (83 mg; 0.3 mmol) was stirred during the addition, over 5 min at room temperature, of $NaBH_3CN$ (10 mg, 0.31 mmol). After 25 min overall, water (5 ml) was added and the mixture was concentrated to half-volume under reduced pressure. The pH was adjusted to 8 by addition of diluted ammonia and extraction with CH_2Cl_2 gave pure *ent-N*(1)-methyl-14,15-didehydroaspidospermidine (**7**).

References

1. S. Pignatti, *Giorn. Bot. Ital.*, **1977**, *111*, 45.
2. D. Stauffacher, *Helv. Chim. Acta* , **1961**, *44*, 2006.
3. B. Pyuskyulev, I. Ognyanov, and P. Panov, *Tetrahedron Lett.*, **1967**, 4559.
4. T. A. Henry, *J. Chem. Soc.*, **1932**, 2759.
5. B. Gilbert, J. A. Brissolese, N. Finch, W. I. Taylor, H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **1963**, *85*, 1523.
6. I. Ognyanov, B. Pyuskyulev, I. Kompiš, T. Sticzay, G. Spiteller, M. Shamma, and R. J. Shine, *Tetrahedron*, **1968**, *24*, 4641.
7. J. L. Pousset and J. Poisson, *Compt. Rend.*, **1964**, *259*, 597.
8. K. Biemann, M. Friedmann-Spiteller, and G. Spiteller, *J. Am. Chem. Soc.*, **1963**, *85*, 631.
9. A. A. Gorman and H. Schmid, *Monatsh. Chem.*, **1967**, *97*, 1554.
10. M. Plat-Berry, J. Le Men, M. M. Janot, T. M. Wilson, H. Budzikiewicz, L. J. Durham, Y. Nakagawa, and C. Djerassi, *Tetrahedron Lett.*, **1962**, 271.
11. W. Klyne, R. J. Swan, B. W. Bycroft, D. Schumann, and H. Schmid, *Helv. Chim. Acta*, **1965**, *48*, 443.
12. M. Hesse, "Indolalkaloide", ed. by H. Budzikiewicz, Verlag Chemie, Vol. I, 1974.
13. A. Ahond, M. M. Janot, N. Langlois, G. Lukacs, P. Potier, P. Rasoanaivo, M. Sangare', N. Neuss, M. Plat, J. Le Men, E. W. Hagaman, and E. Wenkert, *J. Am. Chem. Soc.*, **1974**, *96*, 633.
14. W. Klyne, R. J. Swan, A. A. Gorman, A. Guggisberg, and H. Schmid, *Helv. Chim. Acta*, **1968**, *51*, 1168.
15. A. A. Gorman, N. J. Dastoor, M. Hesse, W. von Philipsborn, V. Renner, and H. Schmid, *Helv. Chim. Acta*, **1969**, *52*, 33.
16. C. Riche and C. Pascard-Billy, *Acta Cryst.*, **1975**, *B 32*, 1975.
17. P. Rasoanaivo, N. Langlois, and P. Potier, *Tetrahedron Lett.*, **1974**, 3669.

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