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# INDOLE ALKALOIDS FROM AERIAL PARTS OF *VINCA SARDOA*

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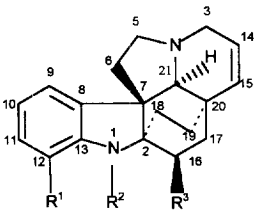
**Key Word Index**—*Vinca sardoa*; Apocynaceae; plumerane alkaloids; indole alkaloids; $^{13}C$ NMR.

**Abstract**—From the aerial parts of *Vinca sardoa*, seven indole alkaloids were isolated, conoflorine and six plumerane-type indolines, *N*(1)-methyl-14,15-didehydro-12-hydroxyaspidofractinine, *N*(1)-methyl-14,15-didehydro-12-methoxyaspidofractinine, *N*(1)-formyl-14,15-didehydroaspidofractinine, *N*(1)-formyl-14,15-didehydro-12-hydroxyaspidofractinine and the known, venalstonine and *N*(1)-methyl-14,15-didehydroaspidofractinine. © 1997 Elsevier Science Ltd

## INTRODUCTION

*Vinca sardoa* (Stearn) Pign. ( = *V. difformis* Pourret ssp. *sardoa*) is a herbal plant native of Sardinia [1]. From its roots, 10 alkaloids were isolated, the known norfluorocurarine, akuammigine, carapanaubine, majdine, isomajdine, rauvoxinine and four new *N*-methylindolines, *vis.*, *ent*-*N*(1)-methyl-14,15-didehydroaspidospermidine, *N*(1)-methyl-14,15-didehydroaspidofractinine, **1**, *N*(1)-methylaspidofractinine and *N*(1)-methyl-14,15-didehydrotuboxenine [2].

The isolation and identification of four new 14,15-didehydroaspidofractinine derivatives, **2**–**5**, besides **1**, conoflorine [3] and venalstonine **6** [4] from the aerial parts of *V. sardoa* are reported here.



| | R¹ | R² | R³ | |
|---|---|---|---|---|
| 1 | H | Me | H | N(1)-methyl-14,15-didehydroaspidofractinine |
| 2 | OH | Me | H | N(1)-methyl-14,15-didehydro-12-hydroxyaspidofractinine |
| 3 | OMe | Me | H | N(1)-methyl-14,15-didehydro-12-metoxyaspidofractinine |
| 4 | H | CHO | H | N(1)-formyl-14,15-didehydroaspidofractinine |
| 5 | OH | CHO | H | N(1)-formyl-14.15-didehydro-12-hydroxyaspidofractinine |
| 6 | H | H | COOH | venalstonine |

## RESULTS AND DISCUSSION

The basic extract of the aerial parts of *V. sardoa* (0.22%) was submitted to counter-current distribution (CCD) between dichloromethane and buffer at discontinuously decreasing pH. Seven alkaloids, **1**–**6** and conoflorine, were isolated.

The most polar compound, **2**, $C_{20}H_{24}N_2O$, is a hydroxy derivative at the aromatic ring of **1**. The $^1H$ and $^{13}C$ NMR data (see Table 1) of the aliphatic moiety of **1** [2] and **2** showed a close similarity, whereas for **2** the mass spectral peaks including the aromatic moiety (*m/z* 308 $[M]^+$, 280, 188 and 174) showed an increase of 16 mu with respect to **1**. The absence in the $^{13}C$ NMR spectrum of **2** of the signal of C(12) ($\delta$ 107.6 in **1**) suggested this position for the hydroxyl group, also in agreement with the upfield shifts of *ortho*- and *para*-carbons to C(12) with respect to **1**.

In the CD curve of **2**, the strong positive Cotton effect at 259 nm due to $\pi \rightarrow \pi^*$ transition of the indolinic chromophore accounted for the absolute configuration 7*S*, typical of the normal series and not of the *ent*-series of aspidospermidine [5]; **2** is therefore unambiguously *N*(1)-methyl-14,15-didehydro-12-hydroxyaspidofractinine. The other alkaloids of the CCD separation are in order of decreasing polarity, **6** identified as venalstonine [4], **3**, **1**, identified as *N*(1)-methyl-14,15-didehydroaspidofractinine [2], **4**, conoflorine [3] and **5**.

The $^1H$ and $^{13}C$ NMR signals of **3**, $C_{21}H_{26}N_2O$, $[M+1]^+$ at *m/z* 323, showed a close similarity to those

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Table 1. $^{13}C$ NMR data of compounds **1–5**

| Carbon | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Position | | | | | |
| 2 | 68.5 | 68.5 | 68.5 | 66.3 | 67.2 |
| 3 | 49.5 | 49.4 | 49.5 | 48.9 | 49.0 |
| 5 | 50.5 | 50.9 | 50.5 | 50.4 | 50.3 |
| 6 | 36.2 | 36.1 | 36.2 | 36.5 | 36.1 |
| 7 | 55.4 | 55.7 | 55.4 | 56.0 | 56.3 |
| 8 | 139.2 | 138.0 | 139.2 | 140.0 | 135.1 |
| 9 | 120.8 | 116.2 | 111.8 | 117.5 | 112.8 |
| 10 | 117.9 | 119.6 | 120.8 | 121.5 | 117.4 |
| 11 | 126.9 | 114.0 | 107.6 | 124.5 | 113.0 |
| 12 | 107.6 | 142.4 | 146.8 | 124.4 | 155.2 |
| 13 | 151.7 | 143.0 | 141.5 | 141.0 | n.o. |
| 14 | 126.2 | 126.0 | 126.9 | 127.3 | 128.3 |
| 15 | 134.0 | 133.9 | 133.9 | 132.7 | 132.6 |
| 16 | 22.6 | 23.3 | 22.6 | 25.4 | 25.6 |
| 17 | 24.4 | 24.0 | 24.0 | 25.2 | 26.8 |
| 18 | 29.0 | 29.1 | 28.9 | 29.0 | 28.7 |
| 19 | 30.1 | 30.3 | 29.9 | 30.0 | 29.7 |
| 20 | 34.3 | 34.1 | 34.2 | 34.6 | 34.6 |
| 21 | 67.3 | 67.8 | 67.3 | 66.4 | 66.2 |
| N-Me | 29.4 | 32.1 | 31.9 | | |
| O-Me | | | 56.1 | | |
| N-CHO | | | | 158.0 | 157.8 |

n.o. = not observed.

of **2**, except for the presence of an additional methyl group, $\delta$(H) 3.74(*s*) and $\delta$($^{13}$C) 56.1, linked at the downfield aromatic C(O), $\delta$ 146.8, C(12). This substitution is in agreement with the upfield shifts of *ortho* C(11) and *para* C(9) ($\delta$ 107.6 and 111.8, respectively, compared with $\delta$ 114.0 and 116.2 in **2**). Alkaloid **3** is therefore *N*(1)-14,15-didehydro-12-methoxyaspidofractinine.

Compound **4** corresponds to the empirical formula $C_{20}H_{22}N_2O$, $[M+1]^+$ at *m/z* 307. Differently from **2** and **3**, its aromatic moiety is not substituted and its $^1$H and $^{13}$C NMR signals are different from the corresponding ones of **1**. In particular, in **4**, H-12 is shifted markedly downfield ($\delta$ 8.0, *d*, *J* = 8.1 and 2.0 Hz) and this can be related to the replacement at *N*(1) of the methyl by a formyl ($\delta$(H) 8.30(*s*); $\delta$($^{13}$C) 158.0). The carbonyl substituent in the *cis* conformation deshields the *peri* H-12. The positive Cotton effect in **4** at 260 nm assigned a 7*S* configuration to this *N*-acylindoline. The close similarity of the non-aromatic $^{13}$C NMR signals of **1** and **4** (Table 1) suggested therefore the structure *N*(1)-formyl-14,15-didehydroaspidofractinine for **4**.

Akaloid **5**, $C_{20}H_{22}N_2O_2$, $[M+1]^+$ at *m/z* 323, is the 12-hydroxy derivative of **4**. Their aliphatic NMR signals are similar, whereas the deshielded H-12 of **4** is lacking and it is replaced in **5** by a hydroxyl group, $\delta$(H) 10.59 (*s*); $\delta$ $^{13}$C(12) 155.2.

## EXPERIMENTAL

A Craig Post apparatus (200 stages, 10:10 ml, upper and lower phase) was used for sepns by CCD. NMR spectra were recorded in $CDCl_3$ at 500 and 125 MHz, respectively, for $^1$H and $^{13}$C using TMS as int. standard.

*Plant material, extraction and separation. Vinca sardoa* collected in Iglesias (Sardinia) was identified by F. Poli and a voucher sample is deposited at the Herbarium of Orto Botanico, Cagliari. Aerial parts (1.2 kg) coarsely minced were submitted to repeated extraction with 2% aq. HOAc. The combined solns were made alkaline with $NaHCO_3$ and exhaustively extracted with $CH_2Cl_2$. The residue of the organic solvent (2.6 g, 0.22%) was submitted to CCD using $CH_2Cl_2$ as stationary phase and phosphate-citric acid buffer at discontinuously decreasing pH as mobile phase. At pH 5.4, *N*(1)-methyl-14,15-didehydro-12-hydroxyaspidofractinine, ($K_r \cdot K_b = 1.5 \times 10^{-9}$, 56 mg) was eluted and then at pH 3.4, in the order, venalstonine, **6** ($K_r \cdot K_b = 10^{-11}$, 87 mg), *N*(1)-methyl-14,15-didehydro-12-methoxyaspidofractinine, **3** ($K_r \cdot K_b = 6 \times 10^{-12}$, 215 mg) and *N*(1)-methyl-14,15-didehydroaspidofractinine, **1** ($K_r \cdot K_b = 4 \times 10^{-12}$, 26 mg). Finally, at pH 2.8 *N*(1)-formyl-14,15-didehydroaspidofractinine, **4** ($K_r \cdot K_b = 2 \times 10^{-12}$, 108 mg), conoflorine ($K_r \cdot K_b = 10^{-12}$, 72 mg) and *N*(1)-formyl-14,15-didehydro-12-hydroxyaspidofractinine, **5** ($K_r \cdot K_b = 8 \times 10^{-13}$, 43 mg) were eluted consecutively. Venalstonine [14] **6**, and conoflorine [3] were identified by their spectroscopic data and by comparison with lit.

*N*(1)-*Methyl*-14,15-*didehydro*-12-*hydroxyaspidofractinine* (**2**). Amorphous. EI-MS, *m/z* (rel. int.): 308, ($[M]^+$, $C_{20}H_{24}N_2O$, 12), 280 ($[M-C_2H_4]^+$, 9), 188(100), 174(21), 135(44), 122(19), 107(52). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 214(4.30), 253(3.76), 292(3.46); $[\alpha]_D^{23} = +98.4°$C (MeOH, *c* 0.23). $^1$H NMR: $\delta$ 3.51 (*dt*, *J* = 16.5 and 2.3 Hz, $H_a$-3), 3.39 (*ddd*, *J* = 16.5, 4.0 and 2.3 Hz, $H_b$-3), 6.74, (*dd*, *J* = 8.0 and 2.1 Hz, H-9), 6.5–6.6 (H-10, H-11, overlapped), 5.65 (*ddd*, *J* = 10.1, 4.0 and 2.3 Hz, H-14), 5.50 (*dt*, *J* = 10.1 and 2.3 Hz, H-15), 2.83 (*s*, N-Me). CD: [$\Theta$], MeOH: $+15.6 \times 10^3$ (259 nm).

*N*(1)-*Methyl*-14,15-*didehydro*-12-*methoxyaspidofractinine* (**3**). Mp 138–140°C (EtOAc-cyclohexane). CI-MS, *m/z* (rel. int.): 323 ($[M+1]^+$, $C_{21}H_{26}N_2O$, 100), 293 ($[M+1]^+$-$OCH_2$, 14), 202(12), 135(9). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$); 211(4.35), 257(3.96), 295(3.56). $[\alpha]_D^{23} = +159°$ (MeOH, *c* 0.13), $^1$H NMR: $\delta$ 3.49 (*dt*, *J* = 16.5 and 2.1 Hz, $H_a$-3), 3.39 (*ddd*, *J* = 16.5, 4.1 and 2.1 Hz, $H_b$-3), 6.71 (*dd*, *J* = 8.0 and 2.1 Hz, H-9), 6.70 (*t*, *J* = 8.0 Hz, H-10), 6.79 (*dd*, *J* = 8.0 and 2.1 Hz, H-11), 5.64 (*ddd*, *J* = 10.1, 4.1 and 2.1 Hz, H-14), 5.50 (*dt*, *J* = 10.1 and 2.1 Hz, H-15), 2.86 (*s*, N-Me), 3.74 (*s*, O-Me). CD: [$\Theta$], MeOH: $+29 \times 10^3$ (262 nm).

*N*(1)-*Formyl*-14,15-*didehydroaspidofractinine* (**4**). Mp 138–140° (cyclohexane). CI-MS, *m/z* (rel. int.): 307 ($[M+1]^+$, $C_{20}H_{22}N_2O$, 100), 281(14). UV $\lambda_{max}^{MeOH}$

nm (log $\varepsilon$): 211 (4.34), 254 (4.13), 278 (3.76), 287 (3.70). $[\alpha]_D^{23} = +55.8°$ (MeOH, *c* 0.5). $^1$H NMR: $\delta$ 3.51 (*dt*, $J = 16.1$ and 2.3 Hz, $H_a$-3), 3.39 (*ddd*, $J = 16.1$, 4.0 and 2.3 Hz, $H_b$-3), 7.0–7.2 (H-9, H-10, H-11), 8.00 (*dd*, $J = 8.1$ and 2.0 Hz, H-12), 5.66 (*ddd*, $J = 10.1$, 4.0 and 2.3 Hz, H-14), 5.49 (*dt*, $J = 10.1$ and 2.3 Hz, H-15), 8.30 (*s*, CHO). CD: [$\Theta$], MeOH: $+28 \times 10^3$ (260 nm).

*N*(1)-*Formyl*-14,15-*didehydro*-12-*hydroxyaspidofractinine* (**5**). Mp 165° (dec.) (cyclohexane). CI-MS, *m/z* (rel. int.): 323 ($[M+1]^+$, $C_{20}H_{22}N_2O_2$, 100), 305 (7), 253 (11), 161 (11). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 216 (4.41), 256 (3.94), 286 (3.69). $[\alpha]_D^{20} = -39°$ (MeOH, *c* 0.3). $^1$H NMR: $\delta$ 3.47 (*dt*, $J = 16.1$ and 2.3 Hz, $H_a$-3), 3.39 (*ddd*, $J = 16.1$, 4.0 and 2.3 Hz, $H_b$-3), 6.80 (*dd*, $J = 8.0$ and 2.1 Hz, H-9), 7.04 (*t*, $J = 8.0$ Hz, H-10), 6.71 (*dd*, $J = 8.0$ and 2.1 Hz, H-11), 5.67 (*ddd*, $J = 10.1$, 4.0 and 2.3 Hz, H-14), 5.48 (*dt*, $J = 10.1$ and 2.3 Hz, H-15), 8.06 (*s*, CHO), 10.59 (*s*, OH).

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