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Two macrocyclic spermine alkaloids from *Aphelandra fuscopunctata* (Acanthaceae)☆

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Abstract

Using HPLC-UV(DAD)-MS the MeOH/H_2O/HOAc extract of the roots of *Aphelandra fuscopunctata* (Acanthaceae) was analyzed. The known alkaloid (+)-aphelandrine and two new macrocyclic spermine alkaloids of the (+)-aphelandrine type were isolated, namely (+)-N(6)-hydroxy-aphelandrine and (+)-N(6)-acetoxy-aphelandrine. Their constitutions and absolute configurations were established. (+)-N(6)-Hydroxy-aphelandrine was obtained from (+)-aphelandrine and (+)-N(6)-acetoxy-aphelandrine. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Aphelandra fuscopunctata*; Acanthaceae; Roots; Polyamine; Spermine; Macrocyclic alkaloids; Hydroxylamine; Aphelandrine; HPLC-MS; LC-MS; Mass spectrometry; APCI-MS; ESI-MS

1. Introduction

The plant *Aphelandra fuscopunctata* Markgr. belongs to the family Acanthaceae and is endemic in the department of Huila, Colombia. The genus *Aphelandra* is distributed from southern Mexico to northern Argentina. It is found at elevations between sea level and 4000 m. There are about 165 species known. The roots of several members of this genus (*A. tetragona*, *A. squarrosa*, *A. sinclairiana*, *A. pepe-parodii*, *A. aurantiaca*, *A. chamissoniana*) contain the macrocyclic spermine alkaloid (+)-aphelandrine (**1**) (Bosshardt, Guggisberg, Johne & Hesse, 1978; Dätwyler, Bosshardt, Bernhard & Hesse, 1978; Dätwyler, Bosshardt, Johne & Hesse, 1979; Guggisberg, Prewo & Hesse, 1986). Its biosynthesis, accumulation, and metabolism have been investigated (Papazoglou et al., 1991; Werner, Hedberg, Lorenzi-Riatsch & Hesse, 1993; Werner, Petrini & Hesse, 1997). The number of known macrocyclic spermine alkaloids has increased in the last few years. The alkaloids (−)-*O*-methylorantine from *Chaenorhinum minus* and *Ch. villosum* (Scrophulariaceae) (Bosshardt et al., 1976, 1978; Dätwyler et al., 1979; Zhu & Hesse, 1988) and (−)-orantine (*Ch. minus, Schweinfurthia papilionacea* (Scrophulariaceae), and *Ephedra* plants (Ephedraceae)) (Ahmad & Sultana, 1990; Tamada, Endo & Hikino, 1979; Zhu & Hesse, 1988) have the same constitution as (+)-aphelandrine, except that of (−)-*O*-methylorantine has a different substituent at C(31) (OCH_3 instead of OH), and both compounds have the inverse configuration at C(17) and C(18). The other alkaloids with a similar backbone to (+)-aphelandrine are (−)-ephedradine B (Tamada et al., 1979; Zhu & Hesse, 1988), (−)-ephedradine C (Konno, Tamada, Endo & Hikino, 1980; Zhu & Hesse, 1988), (−)-ephedradine D (Hikino, Ogata & Konno, 1982), and (−)-epi-orantine (Ahmad & Sultana, 1990).

The on-line coupling of HPLC to UV(DAD) and

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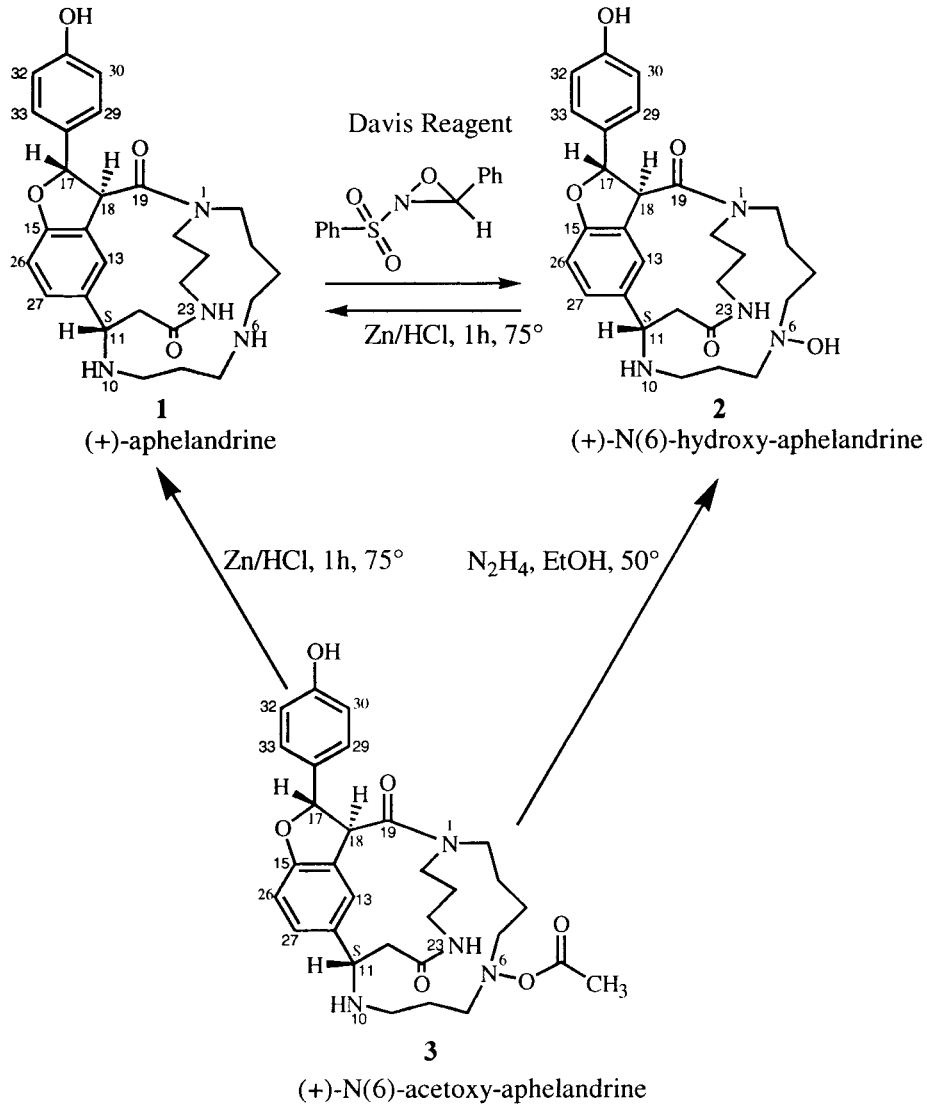

Fig. 1. Chemical correlation of the alkaloids **1**, **2** and **3**.

mass spectrometric detectors is very useful for the quick analysis of complex mixtures from natural sources. This technique allows a fast identification of known components of natural origin and the initial structural investigations of the unknown components, which can be specifically isolated for further investigations by conventional analytical methods.

During our investigations of the alkaloidal content of the roots of *A. fuscopunctata*, the MeOH–H_2O–HOAc extract of the roots was analyzed by HPLC with diode array (DAD) and atmospheric pressure chemical ionisation mass-spectrometric (APCI-MS) detection. Using this HPLC-UV(DAD)-APCI/MS technique we found (+)-aphelandrine and two new macrocyclic alkaloids of the aphelandrine type, which have not been reported so far, namely, (+)-N(6)-hydroxy-aphelandrine (**2**) and (+)-N(6)-acetoxy-aphelandrine (**3**) Fig. 1. In this study we report their constitutions and absolute configurations, which were elucidated by ^{1}H, ^{13}C, and 2D NMR analyses (HMBC, HSQC, and TOCSY experiments), MS, CD, some chemical transformations, and X-ray crystallography.

2. Results and discussion

Two fractions were obtained from the MeOH–H_2O–HOAc (87:10:3) extract of the lyophilized roots of *A. fuscopunctata* after extraction procedures and evaporation of the extract; see Experimental. The HPLC-APCI-MS and TLC analyses showed that both fractions contained the same components, but in different proportions.

2.1. On-line identification of the known component

Fraction II contains predominantly (over 75%) one component. Its data obtained on-line, namely R_t 11.0 min, UV spectrum (λ_{max} = 280 nm), quasi-molecular ion $[M+H]^+$ m/z = 493, and CID (collision induced dissociation) spectrum, after comparison with those from our in-house library, allowed its identification as the known alkaloid (+)-aphelandrine. This was confirmed after the subsequent isolation of the compound and comparison of its TLC behavior (R_f 0.28 mobile phase (S1)) and spectral data (NMR, IR, and CD) with those of (+)-aphelandrine (**1**).

2.2. On-line analysis of the unknown components

The HPLC-UV(DAD)-APCI/MS analysis of fraction I showed that it contains mainly (+)-aphelandrine (**1**) R_t = 11.0 and three other substances with R_t = 14.2 (unknown, currently under investigation), 14.8 **2**, and 17.7 min **3**. The UV spectra of the components **3** and **2** were identical with that of (+)-aphelandrine (λ_{max} = 280 nm) and they have quasi-molecular ions $[M+H]^+$ m/z = 551 and 509, respectively. The on-line CID spectra of these two compounds showed m/z 491 as the first fragmention peak, which corresponds to $[M+H\text{-}60]^+$ and $[M+H\text{-}18]^+$, respectively. This could be interpreted as a neutral loss of HOAc and H_2O, respectively.

2.3. Isolation

The preliminary isolation was achieved by a prep TLC (see Experimental). The band with R_f 0.4–0.6 was scraped off, the substances isolated and afterwards separated by prep HPLC. The fractions obtained by this procedure were evaporated under vacuum. The residues were dissolved in $CHCl_3$ and washed with 5% Na_2CO_3 and H_2O to get the free bases, which were then used for the subsequent analyses.

2.4. Structure elucidation of *3*

The substance **3** has R_t 17.7 min (HPLC), R_f 0.53 (TLC, S1) and M_r = 550 (APCI-MS m/z 551). It gives a positive reaction with Schlittler reagent (Schlittler & Hohl, 1951) (evidence for amino and/or amido groups) and a negative Fluram® test reaction (Weigele, Blount, Tengi, Czaijkowski & Leimgruber, 1972; Weigele, DeBernardo, Tengi & Leimgruber, 1972) (absence of primary amino groups). The number of exchangeable protons is three (ESI/MS ($[M+D]^+$ m/z 555 in MeOH-d_4), which is one less than for (+)-aphelandrine (**1**). The IR spectrum of substance **3** shows a strong absorption at 1760 cm^{-1}, which is absent in the IR spectrum of (+)-aphelandrine. The aromatic region

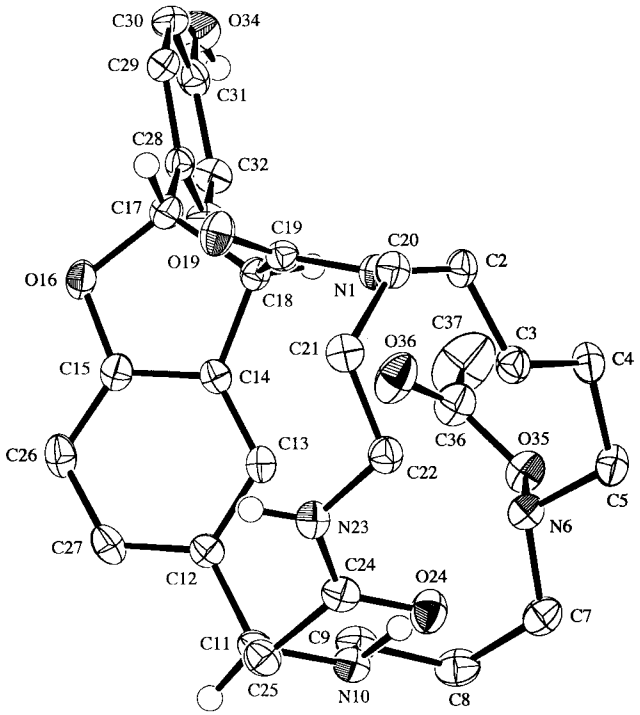

Fig. 2. ORTEP plot of the molecular structure of (+)-N(6)-acetoxy-aphelandrine (**3**) with 50% probability ellipsoids. Most of the H-atoms have been omitted for clarity.

(6.3–8.0 ppm) as well as two signals of the dihydrofurane ring (4.5–6.3 ppm) of the ^1H NMR spectrum (DMSO-d_6) are very similar to that of (+)-aphelandrine. The 3-H singlet at 1.9 ppm is that of the additional OAc group. The protons of this methyl group correlated in the ^1H–^{13}C HSQC spectrum with one C atom, whose signal appears at 19 ppm, and in the ^1H–^{13}C HMBC spectrum with a C atom from a carbonyl group at 170.1 ppm (usual shifts for an OAc group). The above-mentioned fragment ion at m/z = 491 in the APCI/MS/MS spectrum of compound **3**, namely the $[M+H\text{-}60]^+$ ion indicates the presence of an OAc group which is attached to a heteroatom of (+)-aphelandrine (**1**). Furthermore, chemical reduction of compound (**3**) with Zn/HCl (75°, 1 h) leads to **1**. The possible substitution positions in **1** are at the atoms N(6), N(10), N(23). The singlet at 7.48 ppm in the ^1H NMR spectrum of **3** corresponds to the amidic proton at N(23), which excludes additional substitution at this atom. The benzylic proton at C(11) in compound **3** appears in the 1H NMR spectrum at 3.87 ppm, which is normal for (+)-aphelandrine related structures. This suggests that the OAc substituent is at the N(6) atom, and that compound **3** should be (+)-N(6)-acetoxy-aphelandrine. The final confirmation for these conclusions was delivered by the X-ray crystallographic analysis, which also showed that (+)-N(6)-acetoxy-aphelandrine has the same relative configurations at C(17), C(18), and C(11) as (+)-aphe-

landrine. The ORTEP plot (Johnson, 1976) of the molecular structure of **3** is shown in Fig. 2.

2.5. Structure elucidation of substance 2

Compound **2** with R_t 14.8 min (HPLC) and R_f 0.45 (TLC, S1) gives a positive Schlittler reaction (for amides and amines), a negative Fluram® test (no primary amino groups present), and a positive Tollens reaction (for hydroxylamino group). The M_r is 508 (from APCI/MS ($[M+H]^+$ m/z 509)). As a first CID fragmention peak, compound **2** gives a peak at $[M+H\text{-}18]^+$ m/z 491, which corresponds to the neutral loss of H_2O. Compound **2** has four exchangeable protons (ESI-MS, $[M+D]^+$ m/z 514, in MeOH-d_4). Its UV spectrum is nearly identical with those of (+)-aphelandrine (**1**) and (+)-N(6)-acetoxy-aphelandrine (**3**). As in the case of **3**, the chemical reduction (Zn/HCl, 75°, 1 h) of **2** led to **1**. Comparison of the ^1H NMR spectra (^1H, ^{13}C, and ^1H–^1H TOCSY, ^1H–^{13}C HMBC, and ^1H–^{13}C HSQC experiments) of **2** with those of **1** and **3**, showed no differences in the aromatic region of the spectra nor at H-17, H-18, and H-11. Therefore, the alkaloid **2** has the same backbone as (+)-aphelandrine, but with an additional hydroxy group at N(6) or N(10). Substitution at N(10) can be excluded because the signals of the benzylic proton are not shifted compared with those of (+)-aphelandrine (3.77 ppm). To confirm this assumption, we prepared (+)-N(6)-hydroxy-aphelandrine (**2**). It was obtained from (+)-aphelandrine (**1**) with the Davis reagent ((±)-*E*-2-(phenylsulfonyl)-3-phenyloxaziridine) and by reaction of (+)-N(6)-acetoxy-aphelandrine (**3**) with hydrazine. The oxidation of (+)-aphelandrine with Davis reagent yielded (+)-N(6)-hydroxy-aphelandrine as the major product and two minor products (from HPLC-APCI/MS) with quasi-molecular ions $[M+H]^+$ at m/z 509 and 525, respectively (probably N(10)-hydroxy-aphelandrine and N(6),N(10)-dihydroxy-aphelandrine). The reaction of (+)-N(6)-acetoxy-aphelandrine with hydrazine (EtOH, 50°, 1 h) leads to (+)-N(6)-hydroxy-aphelandrine in quantitative yield. This reaction confirmed that the hydroxy group is located at N(6).

2.6. Absolute configurations of the compound 2 and 3

The structure analysis of **3** by X-ray crystallography shows that it has the same relative configuration as (+)-aphelandrine. Synthetic **2** was obtained from (+)-aphelandrine (**1**) by reaction with Davis reagent and from (+)-N(6)-acetoxy-aphelandrine (**3**) by treatment with N_2H_4. During the reduction (Zn/2N HCl) of **2** and **3** the alkaloid (+)-aphelandrine with known absolute configuration is formed and no change of the configuration at C-11, C-17, and C-18 is observed. To determine the absolute configuration, the CD spectra of **2** and **3** were compared with that of (+)-aphelandrine (**1**). All three alkaloids have the same Cotton effects and the same molar ellipticity. All chemical transformations and CD spectra similarities are only possible when these three alkaloids **1**, **2**, and **3** have the same absolute configuration (11S, 17S, and 18S).

3. Conclusions

The alkaloids **2** and **3** have not been reported so far in the literature. They were isolated from *A. fuscopunctata* and their structures and absolute configurations were determined by chemical correlations with the known alkaloid (+)-aphelandrine, X-ray crystallography, and by comparing their CD spectra with those of (+)-aphelandrine. This investigation confirms that the macrocyclic spermine alkaloids are typical for the genus *Aphelandra*.

4. Experimental

4.1. General

TLC: Kieselgel F_{254} plates 0.2 and 2 mm (Merck), detection UV (254, 366 nm), Schlittler (Schlittler & Hohl, 1951), Tollens, and Fluram® (Weigele et al.,1972a; b) reagents. HPLC: All analytical investigations were carried out with a Waters 626 LC System, Waters 996 Photodiode Array Detector and Waters 600S Controller (Waters Corp.) with a Millennium Chromatography Manager 2010 v.2.15 (Waters). The prep HPLC were carried out with a Dynamax SD-300 Solvent Delivery System and a Dynamax UV-1 Detector (Rainin Instument Company, Inc). MS, LC-MS, and LC-MS/MS: Finnigan TSQ 700 triple stage quadrupole instrument, equipped with a Finnigan APCI or ESI ion source. UV: Perkin-Elmer 555 or Perkin-Elmer Lambda 19/UV/Vis/NIR spectrometer. CD: JASCO J-500 A spectropolarimeter. IR: Perkin-Elmer 297 or Perkin-Elmer 781 spectrophotometer. Recording technique: thin layer. NMR: Bruker AMX-600, ARX-300, or AC-300 spectrometer in DMSO-d_6. The solvent was used as internal standard. X-ray: all measurements were made on a Rigaku AFC5R diffractometer.

4.2. TLC, HPLC, and HPLC-MS and MS conditions

TLC: Mobile phases S1: $CHCl_3$–MeOH–NH_4OH (78:19:3), S2: $CHCl_3$–EtOH–NH_4OH (78:19:3). HPLC: Waters Symmetry™ C_8 column (5 μm, 3.9 × 150 mm); flow rate 0.8 ml min^{-1}; gradient mobile phase: H_2O–CH_3CN containing 1% HOAc; 97:3 → 10 min

85:15 → 15 min 85:15 → 90 min 0:100. Prep HPLC conditions: Macherey-Nagel Nucleosil-C_8 column (7 μm, 250/21 mm); flow rate 10 ml min^{-1}; mobile phase: MeOH–H_2O (2:3, v/v) containing 1% HOAc. MS and HPLC-MS: The APCI operating conditions: positive mode; vaporizer temp.: 450°; corona voltage: 4.5 kV; heated capillary temp.: 220°; sheath gas: N_2 with an inlet pressure of 60 PSI. MS/MS experiments: collision gas Ar with a relative pressure 2.2 to 3.1 mTorr; collision induced dissociation offset (coff): −28 eV. ESI operating conditions: positive mode heated capillary temp.: 200°; sheath gas: N_2 with an inlet pressure of 40 PSI; flow: 3 μl min^{-1}.

4.3. Plant material

All plants originate from *Aphelandra fuscopunctata* plants obtained from the Palmengarten, Frankfurt/Main, Germany in 1977. The plants were subcultivated by vegetative propagation in the greenhouse of our institute. During the flowering period (February 1997), the roots were cut and frozen in liquid nitrogen, lyophilized and stored at −20°.

4.4. Extraction

The roots (496 g) were extracted three times with MeOH–H_2O–HOAc (87:10:3) by stirring for 24 h. After filtration, the filtrates were combined and evaporated under vacuum. The residue was dissolved in 2 l 0.2 N aq. HCl and extracted 3× with 700 ml Et_2O. The water phase was separated and the pH was adjusted with 10% NaOH to pH 8.5. The obtained soln was extracted 3× with 800 ml $CHCl_3$ The chloroform extract was combined, dried over Na_2SO_4, and evaporated under vacuum to give fraction I (300 mg 0.06%). The water phase was further basicified with 10% NaOH to pH 9.86 and again extracted with $CHCl_3$ (3 × 800 ml) to give fraction II (350 mg, 0.071%).

4.5. Isolation

200 mg of fraction I were separated by prep TLC (Kieselgel F_{254}) 2 mm with mobile phase S1. From the band with R_f 0.26–0.3, 32 mg (+)-aphelandrine were obtained. The compounds in the band with R_f 0.4–0.6 were recovered and dissolved in MeOH–$CHCl_3$ (2:1). They were separated by prep HPLC (for the conditions see above). The obtained fractions contained 82 mg **3**, 12 mg **2**, and 8 mg of the unknown compound with R_t 14.2 min.

4.6. (+)-Aphelandrine (*1*)

Colourless crystals; APCI-MS *m/z*: 493 $[M+H]^+$; APCI-MS/MS (coff = −32 eV) (rel. int.): 493 (44), 476 (34), 464 (21), 419 (15), 348 (56), 322 (8), 291 (8), 265 (18), 251 (100), 240 (54), 2212 (25), 198 (48), 171 (13), 155 (39), 129 (37); CD $[\theta]_{202}$ −9000, $[\theta]_{207}$ 0, $[\theta]_{215}$ +25000, $[\theta]_{223}$ +15400, $[\theta]_{239}$ +110000, $[\theta]_{266}$ +3200, $[\theta]_{292}$ +7100, $[\theta]_{313}$ 0 (EtOH, *c* 0.006); ^{1}H NMR (300 MHz, DMSO-d_6, 313 K); δ 9.39 (1H, s, phenolic –OH), 7.38 (1H, s, H-13), 7.18 (2H, d, *J* = 8.6 Hz, H-29 and H-33), 6.95 (1H, d, *J* = 8 Hz, H-27), 6.90 (1H, m, NH), 6.74 (2H, d, *J* = 8.6 Hz, H-30 and H-32), 6.67 (1H, d, *J* = 8 Hz, H-26), 6.12 (1H, d, *J* = 6.8 Hz, H-17), 4.71 (1H, d, *J* = 6.8 Hz, H-18), 3.92 (1H, s, NH), 3.84 (2H, m, CH_2), 3.77 (1H, m, H-11), 3.44 (1H, m, CH_2), 3.12 (1H, m, CH_2), 2.86 (2H, m, CH_2), 2.63 (2H, m, CH_2), 2.55 (1H, m, CH_2), 2.35 (3H, m, CH_2), 2.06 (2H, m, CH_2), 1.80 (2H, m, CH_2), 1.55 (4H, m, CH_2), 1.39 (1H, m, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6, 300 K); δ 25.4, 25.6, 25.9, 28.9, 38.6, 43.9, 46.8, 46.9, 47.9, 48.5, 48.7, 51.9 (C-18), 59.6 (C-11), 85.9, 108.1, 115.3 (C-30 and C-32), 122.9 (C-13), 125.5 (C-14), 127.5 (C-28 and C-32), 127.9 (C-27), 131.1 (C-28), 135.5 (C-12), 157.3 (C-31), 157.7 (C-15), 169.4 (C-24), 170.2 (C-19).

4.7. (+)-N(6)-Acetoxy-aphelandrine (=(+)-N(6)-Acetoxy-17(p-hydroxyphenyl)-16-oxa-1,6,10,23-tetraaza-tetracyclo[9,8,6,$2^{12,15}$,$0^{14,18}$]heptacosa-12,14,26-trien-19,24-dione) (*3*)

Colourless crystals, mp 176-–184° (dec.); $[\alpha]_D^{22}$ +192° ± 8 (EtOH, *c* 0.2); APCI-MS *m/z*: 551 $[M+H]^+$; APCI-MS/MS (coff = −32 eV) (rel. int.): 551 (80), 491 (85), 462 (42), 417 (48), 375 (27), 322 (87), 287 (100), 265 (90), 251 (63), 238 (46), 198 (71), 155 (66), 110 (93), 100 (82), 84 (24); UV λ_{max}^{EtOH} 232, 280, 286 (EtOH, *c* 0.006); CD $[\theta]_{202}$ −11000, $[\theta]_{205}$ 0, $[\theta]_{211}$ +32000, $[\theta]_{223}$ +5100, $[\theta]_{239}$ +90400, $[\theta]_{266}$ +3200, $[\theta]_{291}$ +7100, $[\theta]_{313}$ 0 (EtOH, *c* 0.006); IR ν_{max}^{film} cm^{-1}: 3290, 3010, 2960, 2930, 2875, 2690, 2600, 1760 (ester >CO), 1635 (for two amidic >CO), 1518, 1490, 1460, 1440, 1363, 1275, 1250, 1205, 1180, 1110, 1062, 950, 915, 828, 755, 668; ^{1}H NMR (600 MHz, DMSO-d_6, 313 K): δ 7.56 (1H, s, H-13), 7.48 (1H, m, NH), 7.20 (2H, d, *J* = 8.5 Hz, H-29 and H-33), 6.88 (1H, d, *J* = 7.5 Hz, H-27), 6.70 (2H, d, *J* = 8.5 Hz, H-30 and H-32), 6.56 (1H, d, *J* = 8 Hz, H-26), 6.03 (1H, d, *J* = 5.4 Hz, H-17), 4.79 (1H, d, *J* = 5.4 Hz, H-18), 3.87 (2H, m, H-11 and CH_2), 3.80–3.68 (2H, m), 3.58 (1H, m), 3.37 (1H, m), 3.13 (1H, m), 3.03 (1H, m), 2.97 (1H, m), 2.84–2.71 (2H, m), 2.64 (1H, m), 2.56 (1H, m), 2.27 (1H, m), 2.15 (1H, m) all for CH_2, 1.88 (3H, s, CH_3), 1.67–1.49 (7H, m, aliph. H-3, H-5, H-8, H-21), 1.33 (1H, m, CH_2); ^{13}C NMR (300 MHz, DMSO-d_6, 313 K); δ 19.4 (CH_3), 23.2, 25.8, 26.1, 27.6, 39.3, 42.6, 43.4, 47.7, 49.6 (CH_2), 52.1 (C-18),54.7, 58.4 (C-11), 59.4 (CH_2), 86.2 (C-17),

108.4 (C-26), 115.6 (C-30 and C-32), 124.3 (C-13), 126.5 (C-14), 128.2 (C-27), 128.5 (C-29 and C-33), 132.0 (C-28), 132.5 (C-12), 157.5 (C-15 and C-31), 170.1 (CO–O), 170.8 (CO–N), 171.4 (CO–N); X-ray analysis: crystallized from MeOH–H_2O; $C_{30}H_{38}N_4O_6 \cdot 3H_2O$, $M_r = 604.70$ g mol^{-1}, colourless prisms, orthorhombic, space group $P2_12_12_1$, $a = 11.752(2)$, $b = 25.263(3)$, $c = 10.518(2)$ Å, $V = 3122.4(6)$ Å^3, $Z = 4$, $D_x = 1.286$ g cm^{-3}, $\mu = 0.0951$ mm^{-1}, $T = 173$ K, Rigaku AFC-5R diffractometer, Mo Kα radiation, $\lambda = 0.71069$ Å, no absorption correction, structure solved by direct methods with SIR-92 (Altomare, et al., 1994) and refined with *teXsan* (Molecular Structure Corporation, 1992). Of the 5796 measured reflections ($2\theta < 60°$), 5658 were unique and 4718 reflections [$I > 2\sigma(I)$] were used for the least-squares refinement on F of 553 parameters. Final $R = 0.0383$, $R_w = 0.0318$, $GoF = 1.665$, $\Delta_{max}/\sigma = 0.0004$, $\Delta\rho_{max} = 0.25$ e Å^{-3}. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-113835. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. (fax: +44-1223-336033; email: deposit@ccdc.cam.ac.uk).

*4.8. (+)-N(6)-Hydroxy-aphelandrine (=(+)-N(6)-Hydroxy-17(p-hydroxyphenyl)-16-oxa-1,6,10,23-tetraaza-tetracyclo[9,8,6,2^{12,15},0^{14,18}]heptacosa-12,14,26-trien-19,24-dione) (**2**)*

Colourless crystals, mp 186–190° (dec.); $[\alpha]_D^{21}$ +125° ±8 (EtOH, *c* 0.18); APCI-MS *m/z*: 509 $[M+H]^+$; APCI-MS/MS (coff = −35 eV) (rel. int.): 509 (30), 491 (9), 419 (10), 405 (17), 448 (40), 322 (100), 306 (48), 291 (14), 280 (12), 265 (96), 251 (70), 245 (25), 198 (32), 184 (18), 171 (60), 155 (82), 145 (31), 128 (74), 100 (64), 98 (36), 84 (20); UV λ_{max}^{EtOH} 232, 280, 286 (EtOH, *c* 0.0008); CD $[\theta]_{202}$ −15000, $[\theta]_{205}$, 0 $[\theta]_{209}$ +65000, $[\theta]_{222}$ +15200, $[\theta]_{238}$ +101000, $[\theta]_{266}$ +1500, $[\theta]_{293}$ +10000, $[\theta]_{313}$ 0 (EtOH, *c* 0.0008); IR ν_{max}^{film} cm^{-1}: 3260, 2920, 1640, 1550, 1515, 1490, 1460, 1415, 1355, 1255, 1170, 1115, 955, 830, 750; ^{1}H NMR (300 MHz, DMSO-d_6, 330 K) δ: 9.25 (1H, br s, phenolic –OH), 7.43 (1H, s, H-13), 7.37 (1H, br m, NH), 7.19 (2H, d, J = 8.5 Hz, H-29 and H-33), 7.10 (1H, br s, NH), 6.94 (1H, d, J = 7, H-27), 6.78 (2H, d, J = 8.5 Hz, C-30 and C-32), 6.69 (1H, d, J = 7 Hz, C-17), 4.66 (1H, d, J = 7 Hz, C-18), 3.94 (1H, m), 3.86 (1H, m, H-11), 3.75 (1H, m), 3.38 (1H, m), 3.2–2.7 (2H, m), 2.66–2.40 (4H, m), 2.15 (2H, m), 1.85–1.68 (2H, m), 1.66–1.40 (4H, m); ^{13}C NMR (150 MHz, DMSO-d_6, 350 K); δ: 23.1 (CH_2), 25.9, 26.0, 27.0 (CH_2), 39.5, 43.7, 44.1, 46.0, 48.6 (CH_2), 51.9 (C-18), 56.9 (CH_2), 58.7 (C-11), 59.9 (CH_2), 85.4 (C-17), 107.7 (C-26), 115.0 (C-30 and C-32), 122.8 (C-14), 125.4 (C-14), 126.6 (C-29 and C-33), 127.3 (C-27), 130.9 (C-12), 133.8 (C-28), 156.8 (C-15), 157.2 (C-31), 169.5 (C-24), 169.8 (C-19).

*4.9. (+)-Aphelandrine (**1**) from (+)-N(6)-acetoxy-aphelandrine (**3**)*

To a soln of 10 mg of **3** (0.0182 mmol) in 2 N aq. HCl (4 ml), Zn powder (100 mg) was added. The reaction mixture was heated to 75° and stirred for 1 h. After cooling and filtration, the filtrate was evaporated under vacuum. The residue was chromatographed on silica gel (mobile phase S1) to give 8 mg (89%) of **1**.

*4.10. (+)-Aphelandrine (**1**) from (+)-N(6)-hydroxy-aphelandrine (**2**)*

To a soln of 4 mg (0.0079 mmol) of **2** in 2 N aq. HCl (4 ml), Zn powder (100 mg) was added. The reaction mixture was heated to 75° and stirred for 1 h. After cooling and filtration, the filtrate was evaporated under vacuum. The residue was chromatographed on silica gel (mobile phase S1) to give 3 mg (78%) of **1**.

*4.11. (+)-N(6)-Hydroxy-aphelandrine (**2**) from (+)-N(6)-acetoxy-aphelandrine (**3**)*

To a soln of **3** (5 mg, 0.0091 mmol) in 5 ml EtOH, (200 µl) N_2H_4 was added under stirring at room temp. The reaction mixture was heated at 50° for 1 h. After cooling, the solvent was evaporated under vacuum. The residue was chromatographed on the silica gel with mobile phase S1 to give 4.5 mg (98%) of **2**.

*4.12. (+)-N(6)-Hydroxy-aphelandrine (**2**) from (+)-aphelandrine (**1**)*

To a soln of 25 mg (+)-aphelandrine (0.051 mmol) in 30 ml of $CHCl_3$–MeOH (2:1), a soln of 10.6 mg (±)-(*E*)-2-(phenylsulfonyl)-3-phenyloxaziridine (0.041 mmol = Davis reagent (Vishwakarma, Stringer & Davis, 1987)) in 15 ml $CHCl_3$ was added. The reaction mixture was stirred 5 h at room temp. The solvents were evaporated under vacuum. The residue was chromatographed by silica gel prep TLC with mobile phase S2, followed by prep HPLC separation to give 17 mg (80%) of **2**.

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