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Anhydronium bases from *Rauvolfia serpentina*

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Abstract

Five anhydronium bases were isolated by preparative HPLC from a methanolic extract of *Rauvolfia serpentina* roots. For the first time 3,4,5,6-tetradehydroyohimbine, 3,4,5,6-tetradehydro-(*Z*)-geissoschizol, 3,4,5,6-tetradehydrogeissoschizol and 3,4,5,6-tetradehydrogeissoschizine-17-*O*-β-D-glucopyranoside were isolated from a natural source. In addition, the well-known anhydronium base serpentine was isolated. The structures of the compounds were determined by ^{1}H and ^{13}C NMR, MS and UV.

Keywords: *Rauvolfia serpentina*; Apocynaceae; Structure elucidation; Anhydronium bases; Serpentine; 3,4,5,6-Tetradehydroyohimbine; 3,4,5,6-Tetradehydro-(*Z*)-geissoschizol; 3,4,5,6-Tetradehydrogeissoschizol; 3,4,5,6-Tetradehydrogeissoschizine-17-*O*-β-D-glucopyranoside

1. Introduction

Roots of *Rauvolfia serpentina* (L.) Benth. ex Kurz (Apocynaceae) have been established as a rich source of several indole-type alkaloids. Many indole and dihydroindole alkaloids from this plant have served as leads in developing novel drugs for the treatment of cardiovascular diseases (Weiss and Fintelmann, 1999). Recently the highly basic anhydronium base serpentine (**5**) has been found to exhibit anticancer and antimalarial properties (Beljanski and Beljanski, 1982, 1984, 1986; Wright et al., 1996). Similar to other anhydronium bases its mechanism of action is discussed as DNA-intercalation and topoisomerase II inhibition (Dassonneville et al., 1999). The present study describes the isolation and structural elucidation of four novel anhydronium bases from *R. serpentina* (**1**–**4**) together with the well-known alkaloid serpentine (**5**) (Fig. 1).

2. Results and discussion

Dried roots of *Rauvolfia serpentina* were extracted successively with *n*-pentane, methylene chloride and methanol. The methanolic extract was evaporated and dissolved in water. The pH of the suspension was adjusted to 7.0 and an extraction was performed using methylene chloride. Subsequently the pH of the water phase was adjusted to 10.5 and another extraction using methylene chloride was carried out. The latter methylene chloride extract contained the highly basic alkaloids. These were further separated by repeated preparative HPLC to yield compounds **1**–**5**. To avoid generation of artefacts, all procedures were carried out under mild conditions such as low temperature, subdued light and nearly total exclusion of oxygen. Furthermore analytical HPLC was performed before and after base treatment. No difference in the resulting chromatograms could be observed.

Compounds **1**–**5** exhibit nearly the same UV spectrum with strong absorption maxima at about $\lambda = 252$, 307 and 365 nm. TLC analysis displayed spots with a clear blue fluorescence at 366 nm. The EI-mass spectrum reveals a fragment ion at $m/z = 182$ for all of these compounds. To date serpentine (**5**) and the bisindole alkaloid serpentinine have been the only known alkaloids in *R. serpentina* to exhibit this spectroscopic profile, typical for a quaternary β-carbolinium structure element. Alstonine, a stereoisomer of **5**, occurs predominantly in African and American *Rauvolfia* species and could therefore not be detected in the present species. Compound **5** was fully characterized by means of

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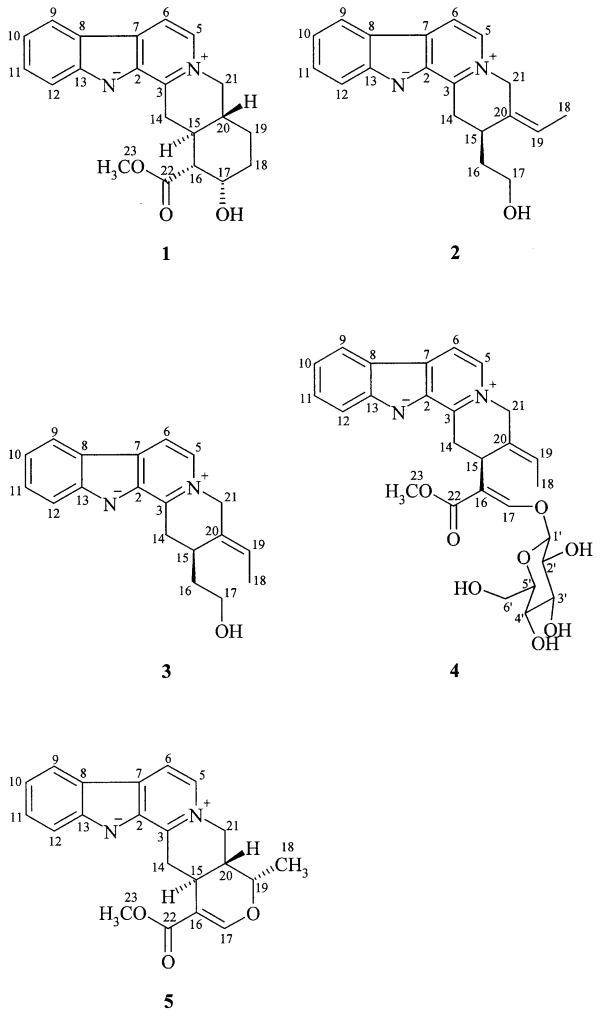

Fig. 1. Structures of the isolated anhydronium bases.

^{1}H and ^{13}C NMR, HMQC and HMBC experiments. Compounds **1**–**4** were characterized in analogy to the methods applied for the structural elucidation of **5**.

Compound **1** shows UV maxima at $\lambda = 252$, 306 and 365 nm. High resolution mass spectrometry of **1** reveals the $[M+H]^+$ peak at $m/z = 351.1748$ suggesting the molecular formula $C_{21}H_{22}N_2O_3$, containing four protons less than yohimbine. The ^{1}H NMR spectrum of **1** exhibits a pair of vicinal protons at δ 8.43 (1H, *d*, $J = 6.6$ Hz) and 8.29 (1H, *d*, $J = 6.6$ Hz) that can be ascribed to the aromatic protons H-6 and H-5, respectively, of the β-carbolinium structure. These and the other aromatic protons at δ 8.32 (1H, *dd*, $J = 8.0$, 1.0 Hz), 7.43 (1H, *ddd*, $J = 8.0$, 7.0, 0.9 Hz), 7.75 (1H, *ddd*, $J = 8.3$, 7.0, 1.0 Hz) and 7.71 (1H, *dd*, $J = 8.3$, 0.9 Hz) have similar shifts and the same multiplicity as given for serpentine (**5**), confirming the presence of a quaternary β-carbolinium function in **1**. This was further supported by identical shifts of the aromatic carbons in the ^{13}C NMR spectra of **1** and **5**. The assignment of the single carbons was determined by DEPT pulse sequence and HMQC experiments.

A three proton singlet at δ 3.81 in the ^{1}H NMR spectrum indicates the presence of a methoxycarbonyl group as the carbon signals in the ^{13}C NMR spectrum at δ 52.4 and 174.3 suggested. The coupling constants of the methylene protons H-14 [δ 4.06 (1H, *dd*, $J = 18.7$, 5.0 Hz) and δ 3.08 (1H, *dd*, $J = 18.7$, 10.1 Hz)] and H-21 [δ 4.78 (1H, *dd*, $J = 13.3$, 4.5 Hz) and δ 4.44 (1H, *dd*, $J = 13.3$, 13.0 Hz)] indicate a *trans* diaxial position of the protons H-15 [δ 2.62 (1H, *dddd*, $J \approx 10.1$, 10.1, 10.1, 5.0 Hz)] and H-20 [δ 2.12 (1H, *m*)]. Despite of complex signal multiplicity and overlap, the protons H-16 at δ 2.62 (1H, *m*) and H-17 at δ 4.44 (1H, *m*) could be ascribed to β-axial and β-equatorial orientations, respectively.

All these data are in good agreement with reported data for 3,4,5,6-tetradehydroyohimbine (**1**) prepared from a synthetic source (Stahl and Borschberg, 1996).

The UV spectrum of compound **2** exhibits maxima at $\lambda = 253$, 308 and 366 nm and its molecular formula was determined as $C_{19}H_{20}N_2O$ by HR-MS. In contrast to **1** no signals of a methoxycarbonyl group could be detected in the ^{1}H and ^{13}C NMR spectra, however resonances of an additional ethylidene side chain appeared in the ^{1}H NMR at δ 5.83 (1H, *qd*, $J = 7.0$, 1.3 Hz) for H-19 and δ 1.86 (3H, *d*, $J = 7.0$ Hz) for H-18. The ^{1}H and ^{13}C NMR spectra reveal signals for aromatic protons and carbons similar to those of **1**. The H,H COSY spectrum of **2** exhibits cross-peaks between H-14 [δ 3.75 (1H, *dd*, $J = 17.0$, 5.8 Hz) and δ 3.50 (1H, *dd*, $J = 17.0$, 6.3 Hz)] and H-15 [δ 3.09 (1H, *m*)], H-15 and H-16 [δ 1.79 (1H, *m*) and δ 1.67 (1H, *m*)] and H-16 and H-17 [δ 3.71 (2H, *t*, $J = 6.4$ Hz)] with the latter protons belonging to a hydroxymethylene group. Furthermore H-18 shows strong interaction with H-19 and weak cross-peaks with H-21 [δ 5.49 (1H, *d*, $J = 16.3$ Hz) and δ 5.45 (1H, *d*, $J = 16.3$ Hz)] and H-15, suggesting the quaternary olefinic carbon C-20 as a connective link between C-15 and C-21. This was confirmed by a HMBC spectrum of **2** displaying interaction between C-20 and H-14, H-21, H-16 and H-18. In the same spectrum the connection of methylene carbons C-14 and C-21 with the aromatic β-carbolinium system could be proven, since H-14 exhibits interaction with C-3 and C-21 with H-5.

Proton H-15 was assumed as α-orientated for biogenetical reasons. The (*Z*)-geometry of the ethylidene side chain was concluded from comparison with alkaloid **3** (see below) and from NOE difference measurements, displaying a strong NOE effect between H-18 and H-21. Thus, alkaloid **2** was identified as 3,4,5,6-tetradehydro-(*Z*)-geissoschizol. This is the first time that isolation and characterization of a 3,4,5,6-tetradehydrogenated geissoschizol derivative was performed.

Alkaloid **3** has the same molecular formula as **2** and the EI-mass spectrum of **3** exhibits the same fragment

ions as in **2** with a gradual decomposition of the hydroxyethylene group at C-15. The ^{1}H and ^{13}C NMR spectra of **3** are closely related to those of **2**, with C-15 upfield shifted by 4.6 ppm and C-19, C-20 and C-21 downfield shifted by 2.0, 1.7 and 5.6 ppm, respectively. The large upfield shift at C-15 as well as the large downfield shift at C-21 from **2** to **3** can be explained by strong γ-steric interaction between H-15 and C-18 in **3**, whereas γ-steric interaction between H-21 and C-18 as in **2** is not possible in **3** (Takayama et al., 1992). These data as well as observation of a pronounced NOE effect between H-18 and H-15 confirm the ethylidene side chain of **3** to have (*E*)-geometry. With the help of further published data existing for geissoschizol derivatives alkaloid **3** could be identified as 3,4,5,6-tetradehydrogeissoschizol (Lounasmaa and Hanhinen, 1999). Thus alkaloid **3** is a geometric isomer of alkaloid **2** and represents a further example for a 3,4,5,6-tetradehydrogenated geissoschizol derivative.

The UV spectrum of compound **4** slightly differs from the ones for the alkaloids described above. Beside the strong UV maxima at $\lambda = 252$, 307 and 366 nm two shoulders at $\lambda = 219$ and 233 nm were detected. High resolution mass spectrometry displayed the $[M+H]^+$ peak at $m/z = 511.2107$ suggesting that compound **4** has the molecular formula $C_{27}H_{30}N_2O_8$. The ^{1}H and ^{13}C NMR spectra unambiguously indicate the presence of a quaternary β-carbolinium function similar to that in compounds **1–3** and **5**.

Similar to **5**, signals of an α,β-unsaturated ester system were detected in the ^{1}H NMR spectrum with a singlet for the protons of a methoxycarbonyl group at δ 3.72 and a singlet at δ 7.82 (1H, *brs*) for the olefinic H-17. The ^{13}C NMR spectrum reveals shifts of δ 31.6, 127.5, 133.7 and 62.4 for the carbons C-15, C-19, C-20 and C-21, respectively, closely related to those of **3**. These findings suggest the same configuration for the ethylidene side chain at C-20 for compound **4**. Observation of a pronounced NOE effect between H-18 and H-15 confirms this conclusion.

The signal at δ 4.77 (1H, *d*, $J = 8.2$ Hz) clearly belongs to an anomeric proton indicating the presence of a glucopyranosyl moiety with β-linkage to C-17 of the aglycon. Further data from ^{1}H and ^{13}C NMR spectra verified glycoside **4** to be 3,4,5,6-tetradehydrogeissoschizine-17-*O*-β-D-glucopyranoside. This is the first time that isolation and characterization of a 3,4,5,6-tetradehydrogenated geissoschizine derivative is described.

Biological activities of the isolated compounds have not been sufficiently investigated. It is of major importance to determine whether the novel anhydronium bases also interfere with DNA and topoisomerase II functions as described for serpentine (**5**) and related anhydronium bases. In addition to the anticancer properties their antimalarial activity should be further investigated.

3. Experimental

3.1. General

NMR spectra were recorded in CD_3OD using a JEOL Eclipse+ 500 spectrometer with TMS as internal standard. UV spectra were acquired with a Shimadzu 2101PC UV–vis photometer and optical rotations with a Jasco DIP-370 digital polarimeter. EI-MS spectra were obtained with a Vacuum Generators VG 7070 H mass spectrometer employing an ionizing energy of 70 eV, ESI-MS spectra were recorded on a Micromass AutoSpec spectrometer. Prep. HPLC was carried out on a Waters prep LC 4000 system with 990 Diode Array Detector.

Solvents for extraction with the quality "extra pure" were purchased from Merck KG, Germany, except *n*-pentane, which was purchased from Riedel-de Haën GmbH & Co. KG, Germany. Solvents for chromatography had the quality "LiChrosolv®" and were purchased from Merck KG, Germany.

3.2. Plant material

Roots of *R. serpentina* were purchased from Finzelberg GmbH & Co. KG, Andernach, Germany. A voucher specimen (No. 2181000) is deposited at the Department of Pharmaceutical Chemistry, University of Marburg, Germany.

3.3. Extraction and isolation

Dried plant material (1.92 kg) was powdered, further crushed in *n*-pentane (ice-cooling) using an Ultra Turrax and percolated successively with *n*-pentane, CH_2Cl_2 and MeOH at room temperature. After evaporation of solvents in vacuo at 30 °C, the methanolic residue (200 g) was redissolved in distilled H_2O, filtered and the pH adjusted to 7.0 using NaOH. This soln. was extracted with CH_2Cl_2 (6×300 ml) to remove residual amounts of alkaloids with weak and medium basicity. To yield the highly basic anhydronium bases the pH of the aq. soln. was adjusted to 10.5 and further extracted with CH_2Cl_2 (6×300 ml). The latter CH_2Cl_2 phase was evaporated to dryness at 30 °C, resulting in 1.80 g of a yellow-brownish material, which was fractionated by prep. HPLC (250×21 mm, Nucleosil® 100-5-C-18HD, 5 μm (Macherey & Nagel), 22 ml min^{-1}, Gradient: 0 min (MeOH–H_2O in addition of TFA 0.1% (freshly prepared): (28:72), 55 min (37:63), 63 min (40:60), 83 min (55:45), 95 min (100:0)). Five fractions, showing UV max at about 252, 307 and 365 nm, were collected [fr. 1 (containing **1**): min 36–39, fr. 2 (**2**): 43–46, fr. 3 (**3**): 47–51, fr. 4 (**4**): 54–57, fr. 5 (**5**): 65–69]. These fractions were further purified by prep. HPLC (250×25 mm, RP-selectB, 15 μm (Merck), 20 ml min^{-1}, with following isocratic MeCN–H_2O systems in addition of TFA 0.1%:

fr. 1, 2 (26:74), fr. 3 (24:76), fr. 4, 5 (28:72)) to afford **1** (7.5 mg), **2** (12.0 mg), **3** (8.2 mg), **4** (4.0 mg) and **5** (24.0 mg).

3.4. 3,4,5,6-Tetradehydroyohimbine (*1*)

Yellow powder (7.5 mg), $[\alpha]_D^{20}$ +175.4° (MeOH; *c* 0.3), UV λ_{max}^{MeOH}nm (log ε): 365 (3.37), 306 (3.97), 252 (4.14). ^{1}H NMR (500 MHz, CD_3OD): δ 8.43 (1H, *d*, *J* = 6.6 Hz, H-6), 8.32 (1H, *dd*, *J* = 8.0, 1.0 Hz, H-9), 8.29 (1H, *d*, *J* = 6.6 Hz, H-5), 7.75 (1H, *ddd*, *J* = 8.3, 7.0, 1.0 Hz, H-11), 7.71 (1H, *dd*, *J* = 8.3, 0.9 Hz, H-12), 7.43 (1H, *ddd*, *J* = 8.0, 7.0, 0.9 Hz, H-10), 4.78 (1H, *dd*, *J* = 13.3, 4.5 Hz, Hβ-21), 4.44 (1H, *dd*, *J* = 13.3, 13.0 Hz, Hα-21), 4.44 (1H, *m*, H-17), 4.06 (1H, *dd*, *J* = 18.7, 5.0 Hz, Hα-14), 3.81 (3H, *s*, H-23), 3.08 (1H, *dd*, *J* = 18.7, 10.1 Hz, Hβ-14), 2.62 (1H, *m*, H-16), 2.62 (1H, *dddd*, *J*≈10.1, 10.1, 10.1, 5.0 Hz, H-15), 2.12 (1H, *m*, H-20), 2.03 (1H, *dddd*, *J*≈13.5, 2.8, 2.8, 2.8 Hz, Hα-18), 1.82 (1H, *dddd*, *J*≈13.5, 13.2, 4.8, 2.2 Hz, Hβ-18), 1.77–1.69 (2H, *m*, H-19). ^{13}C NMR (125 MHz, CD_3OD): Table 1. EI-MS (70 eV): *m*/*z* (rel. int.) 350 $[M]^+$ (100), 293 (18), 260 (4), 247 (5), 233 (9), 221 (16), 182 (6), 69 (21), 41 (31). HR-ESI-MS found 351.1748 $[M+H]^+$; $C_{21}H_{23}N_2O_3$ requires 351.1709 $[M+H]^+$.

3.5. 3,4,5,6-Tetradehydro-(*Z*)-geissoschizol (*2*)

Yellow powder (12.0 mg), $[\alpha]_D^{20}$ +7.0° (MeOH; *c* 0.5), UV λ_{max}^{MeOH}nm (log ε): 366 (3.31), 308 (3.97), 253 (4.13). ^{1}H NMR (500 MHz, CD_3OD): δ 8.50 (1H, *d*, *J* = 6.4 Hz, H-6), 8.47 (1H, *d*, *J* = 6.4 Hz, H-5), 8.36 (1H, *dd*, *J* = 8.0, 1.1 Hz, H-9), 7.78 (1H, *ddd*, *J* = 8.0, 6.9, 1.1 Hz, H-11), 7.74 (1H, *dd*, *J* = 8.0, 1.3 Hz, H-12), 7.45 (1H, *ddd*, *J* = 8.0, 6.9, 1.3 Hz, H-10), 5.83 (1H, *qd*, *J* = 7.0, 1.3 Hz, H-19), 5.49 (1H, *d*, *J* = 16.3 Hz, Hβ-21), 5.45 (1H, *d*, *J* = 16.3 Hz, Hα-21), 3.75 (1H, *dd*, *J* = 17.0, 5.8 Hz, Hα-14), 3.71 (2H, *t*, *J* = 6.4 Hz, H-17), 3.50 (1H, *dd*, *J* = 17.0, 6.3 Hz, Hβ-14), 3.09 (1H, *m*, H-15), 1.86 (3H, *d*, *J* = 7.0 Hz, H-18), 1.79 (1H, *m*, H-16), 1.67 (1H, *m*, H′-16). ^{13}C NMR (125 MHz, CD_3OD): Table 1. EI-MS (70 eV): *m*/*z* (rel. int.) 292 $[M]^+$ (30), 274 (14), 272 (17), 261 (9), 247 (100), 233 (6), 182 (1), 69 (8), 43 (8). HR-EI-MS found 292.1577 $[M]^+$; $C_{19}H_{20}N_2O$ requires 292.1576 $[M]^+$.

3.6. 3,4,5,6-Tetradehydrogeissoschizol (*3*)

Yellow powder (8.2 mg), $[\alpha]_D^{20}$ +30.9° (MeOH; *c* 0.3), UV λ_{max}^{MeOH}nm (log ε): 366 (3.28), 307 (3.92), 253 (4.09). ^{1}H NMR (500 MHz, CD_3OD): δ 8.49 (1H, *d*, *J* = 6.4 Hz, H-6), 8.43 (1H, *d*, *J* = 6.4 Hz, H-5), 8.37 (1H, *dd*, *J* = 8.0, 1.1 Hz, H-9), 7.78 (1H, *ddd*, *J* = 8.0, 6.9, 1.1 Hz, H-11), 7.75 (1H, *dd*, *J* = 8.0, 1.3 Hz, H-12), 7.45 (1H, *ddd*, *J* = 8.0, 6.9, 1.3 Hz, H-10), 5.94 (1H, *qd*, *J* = 6.7, 1.3 Hz, H-19), 5.32 (1H, *d*, *J* = 15.1 Hz, Hβ-21), 5.17 (1H, *d*, *J* = 15.1 Hz, Hα-21), 3.84 (1H, *dd*, *J* = 16.8, 6.3 Hz, Hα-14), 3.75 (2H, *t*, *J* = 6.5 Hz, H-17), 3.56 (1H, *dd*, *J* = 16.8, 5.9 Hz, Hβ-14), 3.44 (1H, *m*, H-15), 1.91 (1H, *m*, H-16), 1.79 (3H, *d*, *J* = 6.7 Hz, H-18), 1.67 (1H, *m*, H′-16). ^{13}C NMR (125 MHz, CD_3OD): Table 1. EI-MS (70 eV): *m*/*z* (rel. int.) 292 $[M]^+$ (42), 274 (75), 272 (45), 260 (56), 247 (100), 233 (13), 219 (4), 182 (4), 69 (6), 43 (6). HR-EI-MS found 292.1582 $[M]^+$; $C_{19}H_{20}N_2O$ requires 292.1576 $[M]^+$.

3.7. 3,4,5,6-Tetradehydrogeissoschizine-17-*O*-β-D-glucopyranoside (*4*)

Yellow powder (4.0 mg), $[\alpha]_D^{20}$ +118.3° (MeOH; *c* 0.1), UV λ_{max}^{MeOH}nm (log ε): 366 (3.34), 307 (4.04), 252 (4.23). ^{1}H NMR (500 MHz, CD_3OD): δ 8.50 (1H, *d*, *J* = 6.3 Hz, H-6), 8.48 (1H, *d*, *J* = 6.3 Hz, H-5), 8.38 (1H, *dd*, *J* = 8.1, 1.2 Hz, H-9), 7.82 (1H, *brs*, H-17), 7.78 (1H, *ddd*, *J* = 8.4, 6.9, 1.2 Hz, H-11), 7.74 (1H, *dd*, *J* = 8.4, 1.2 Hz, H-12), 7.45 (1H, *ddd*, *J* = 8.1, 6.9, 1.2 Hz, H-10), 5.86 (1H, *qd*, *J* = 7.0, 1.3 Hz, H-19), 5.58 (1H, *d*, *J* = 14.0 Hz, Hβ-21), 5.19 (1H, *d*, *J* = 14.0 Hz, Hα-21), 4.77 (1H, *d*, *J* = 8.2 Hz, H-1′), 4.26 (1H, *td*, *J*≈6.5, 1.3 Hz, H-15), 3.86 (1H, *dd*, *J* = 15.7, 6.5 Hz, Hα-14), 3.80 (1H, *dd*, *J* = 11.9, 2.3 Hz, H-6′), 3.72 (3H, *s*, H-23), 3.50 (1H, *dd*, *J* = 11.9, 5.8 Hz, H′-6′), 3.37 (1H, *dd*, *J* = 8.9, 8.5 Hz, H-3′), 3.33 (1H, *ddd*, *J* = 9.4, 5.8, 2.3 Hz, H-5′), 3.30 (1H, *dd*, *J* = 9.4, 8.5 Hz, H-4′), 3.17 (2H, *m*, Hβ-14 & H-2′), 1.59 (3H, *d*, *J* = 7.0 Hz, H-18). ^{13}C NMR (125 MHz, CD_3OD): Table 1. EI-MS (70 eV): *m*/*z* (rel. int.) 260 (3), 246 (100), 231 (20), 182 (1), 69 (2), 41 (7). HR-ESI-MS found 511.2107 $[M+H]^+$; $C_{27}H_{31}N_2O_8$ requires 511.2080 $[M+H]^+$.

Table 1
^{13}C NMR spectral data of compounds **1**–**5** in CD_3OD at 125 MHz

Carbon	1	2	3	4	5
2	135.5	135.8	135.7	135.3	135.8
3	141.6	141.8	141.6	141.9	141.2
5	133.8	133.8	133.0	133.0	134.2
6	116.6	116.9	116.8	116.8	116.8
7	132.4	133.0	133.3	132.7	132.6
8	121.4	121.5	121.5	121.6	121.3
9	123.9	124.1	124.1	124.1	124.0
10	123.1	123.2	123.1	123.1	123.2
11	132.7	133.0	133.0	132.8	132.9
12	113.8	113.9	113.8	113.8	113.9
13	145.2	145.4	145.4	145.4	145.4
14	31.6	31.9	30.8	30.2	31.9
15	31.2	35.3	30.7	31.6	26.0
16	53.5	37.4	37.6	113.9	107.2
17	68.2	60.0	60.0	157.8	156.3
18	32.6	13.2	13.8	14.1	14.2
19	23.0	124.8	126.8	127.5	72.9
20	36.8	131.8	133.5	133.7	38.5
21	61.0	55.1	60.7	62.4	57.6
22	174.3			168.7	168.4
23	52.4			52.1	51.8
1′				105.1	
2′				74.6	
3′				77.9	
4′				71.1	
5′				78.9	
6′				63.1	

3.8. Serpentine (**5**)

Yellow powder (24.0 mg), $[\alpha]_D^{20}$ +273.1° (MeOH; *c* 0.7), UV λ_{max}^{MeOH} nm (log ε): 365 (3.32), 307 (4.00), 251 (4.19). ^{1}H NMR (500 MHz, CD_3OD): δ 8.45 (1H, *d*, *J* = 6.6 Hz, H-6), 8.31 (1H, *d*, *J* = 6.6 Hz, H-5), 8.31 (1H, *dd*, *J* = 8.1, 1.0 Hz, H-9), 7.76 (1H, *ddd*, *J* = 8.4, 6.6, 1.0 Hz, H-11), 7.72 (1H, *dd*, *J* = 8.4, 1.3 Hz, H-12), 7.71 (1H, *d*, *J* = 1.4 Hz, H-17), 7.43 (1H, *ddd*, *J* = 8.1, 6.6, 1.3 Hz, H-10), 4.88 (1H, *dd*, *J* = 13.3, 4.1 Hz, Hβ-21), 4.75 (1H, *qd*, *J* = 6.8, 4.2 Hz, H-19), 4.64 (1H, *dd*, *J* = 18.1, 4.7 Hz, Hα-14), 4.59 (1H, *dd*, *J* = 13.3, 13.0 Hz, Hα-21), 3.82 (3H, *s*, H-23), 3.13 (1H, *dd*, *J* = 18.1, 11.6 Hz, Hβ-14), 3.04 (1H, *dddd*, *J* = 11.6, 11.5, 4.7, 1.4 Hz, H-15), 2.67 (1H, *dddd*, *J* = 13.0, 11.5, 4.2, 4.1 Hz, H-20), 1.34 (3H, *d*, *J* = 6.8 Hz, H-18). ^{13}C NMR (125 MHz, CD_3OD): Table 1. EI-MS (70 eV): *m*/*z* (rel. int.) 348 $[M]^+$ (100), 261 (11), 247 (12), 233 (10), 221 (14), 207 (22), 206 (24), 205 (24), 182 (10), 69 (3), 41 (4). HR-EI-MS found 348.1491 $[M]^+$; $C_{21}H_{20}N_2O_3$ requires 348.1474 $[M]^+$.

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References

Beljanski, M., Beljanski, M.S., 1982. Selective inhibition of in vitro synthesis of cancer DNA by alkaloids of β-carboline class. Exp. Cell Biol. 50, 79–87.

Beljanski, M., Beljanski, M.S., 1984. Three alkaloids as selective destroyers of the proliferative capacity of cancer cells. IRCS Med. Sci. 12, 587–588.

Beljanski, M., Beljanski, M.S., 1986. Three alkaloids as selective destroyers of cancer cells in mice. Oncology 43, 198–203.

Dassonneville, L., Bonjean, K., De Pauw-Gillet, M.C., Colson, P., Houssier, C., Quetin-Leclercq, J., Angenot, L., Bailly, C., 1999. Stimulation of topoisomerase II-mediated DNA cleavage by three DNA-intercalating plant alkaloids: cryptolepine, matadine and serpentine. Biochemistry 38, 7719–7726.

Lounasmaa, M., Hanhinen, P., 1999. Conformational study of geissoschizine isomers and their model compounds. Heterocycles 51, 649–670.

Stahl, R., Borschberg, H.J., 1996. A reinvestigation of the oxidative rearrangement of yohimbane-type alkaloids, part B. Helv. Chim. Acta 79, 1361–1378.

Takayama, H., Watanabe, T., Seki, H., Aimi, N., Sakai, S., 1992. Geissoschizine revisited- definite proof of its stereostructure. Tetrahedron Lett. 33, 6831–6834.

Weiss, R.F., Fintelmann, V., 1999. Lehrbuch der Phytotherapie, 9. Auflage. Hippokrates Verlag, Stuttgart.

Wright, C.W., Phillipson, J.D., Awe, S.O., Kirby, G.C., Warhurst, D.C., Quetin-Leclercq, J., Angenot, L., 1996. Antimalarial activity of cryptolepine and some other anhydronium bases. Phytother. Res. 10, 361–363.