Stereochemistry of Caracurine V, *iso*-Caracurine V, Bisnortoxiferine, and Tetrahydrocaracurine V Ring Systems

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The 3D structures of the *Strychnos toxifera* alkaloids caracurine V and toxiferine I, and of the related *iso*-caracurine V and tetrahydrocaracurine V ring systems were determined by means of NMR spectroscopy and semiempirical calculations. The relative spatial arrangement of the aromatic indole rings is similar in all three "caracurine" ring systems. Opening of both tetrahydrooxepine rings of caracurine V to give the bisnortoxiferine ring skeleton leads to the conformational change of the central eight-membered ring from a crown form to a boat form, resulting in a considerably changed geometry of the whole ring skeleton.

Introduction

Many structurally different compounds belonging to various pharmacological groups have been reported to retard the dissociation of antagonists from the muscarinic acetylcholine receptor.[1] This effect is based on an allosteric modulation of the antagonist−receptor complex, caused by a modulator occupying a site apart from the common antagonist binding area.[2,3] The inhibition of ligand dissociation may result in a receptor subtype-specific increase of ligand binding which opens various therapeutic perspectives such as for the therapy of dementia or pain.[1] Molecular modelling studies have led to a hypothesis of the pharmacophore consisting of two positively charged cationic centers at a distance of approximately 10 Å surrounded by two aromatic ring systems.[4,5] The neuromuscular blocker alcuronium (**1**), which is derived from the *Strychnos toxifera* alkaloid toxiferine I (**2**), is a well-known classical enhancer of antagonist binding to muscarinic M_2 receptors.[6] Its double cyclization product, diallylcaracurine V (**3**), has been identified as an allosteric agent with a similarly high allosteric binding affinity.[7]

The stereochemistry of the symmetrical caracurine V ring system (twofold symmetry axis) has been determined by NMR spectroscopy and semiempirical calculations.[8] Opening of both tetrahydrooxepine rings of caracurine V (**4**) to give the bisnortoxiferine ring skeleton would probably result in a conformational change of the central eight-membered ring. Consequently, the relative spatial arrangement of the pharmacophoric elements (two positively charged nitrogens and two aromatic rings), which is crucial for the receptor−ligand interactions, is likely to be different in both ring systems. The different position of the pharmacophoric elements is probably responsible for the different binding properties of alcuronium and diallylcaracurine V at muscarinic M_2 receptors.[9] Our group recently reported the synthesis of *iso*-caracurine V (**5**), which is a one-sided ring-opened product of caracurine V or, alternatively, a one-sided ring-closed product of bisnortoxiferine (Figure 1).[10]

In this paper, we compare the 3D structures of the bisnortoxiferine and *iso*-caracurine V ring systems to that of caracurine V. Additionally, the novel tetrahydrocaracurine V ring system (**6**) has been included in our studies. In all investigated compounds, the pharmacophoric elements are incorporated into highly fused and rigid ring systems. However, depending on the ring skeleton, their relative spatial arrangement could be considerably different. Future 3D quantitative structure-activity relationship studies on derivatives of the ring systems under investigation should reveal the optimal relative position of the aromatic rings and the cationic centers, and thus improve the existing pharmacophore.

Results and Discussion

Synthesis

The calabash-curare alkaloid toxiferine I (**2**) was prepared by the dimerization of Wieland−Gumlich aldehyde methochloride using pivalic acid according to the procedure of Battersby and Hodson.[11] The previously unknown NMR spectra of **2** were assigned by means of HH-COSY, ROESY, HMQC, and HMBC experiments. *iso*-Caracurine V (**5**) was synthesized by a one-sided intramolecular alcohol

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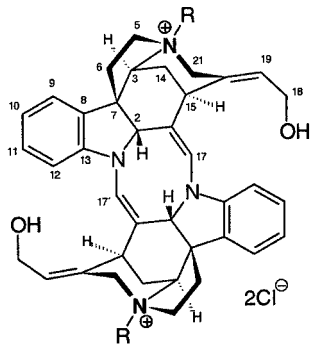
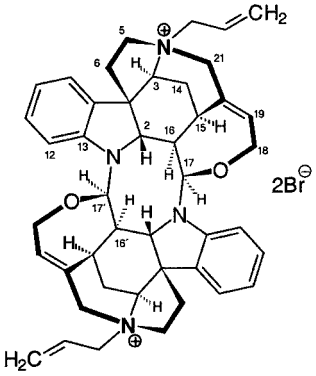
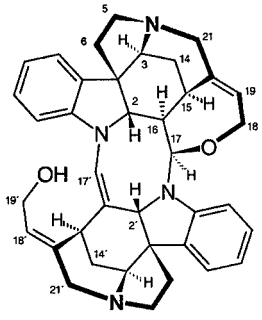


Toxiferine I **2**, R = methyl
Alcuronium **1**, R = allyl



Diallylcaracurine V **3**
Caracurine V base **4**

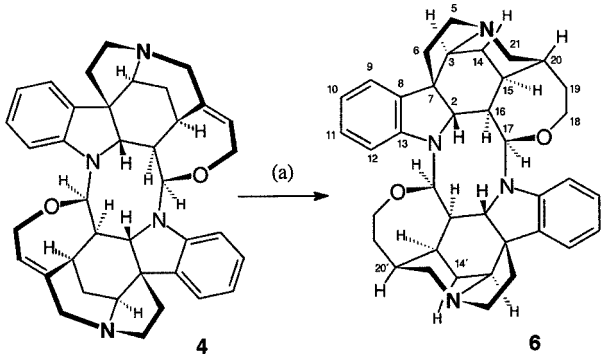


iso-Caracurine V **5**

Figure 1. Structures of the investigated ring systems

elimination from the central diazocane ring of caracurine V using trifluoroacetic acid.[10] 19,20,19′,20′-Tetrahydrocaracurine V (**6**) was obtained by catalytic hydrogenation of **4** in ethanol using PtO_2 as catalyst (Scheme 1).

The single sets of signals in both the ^1H and ^{13}C NMR spectra of **6** show that the twofold symmetry axis is maintained in the course of the reaction. Moreover, addition of hydrogen to both double bonds proceeded stereoselectively from the less-hindered side of the concavo-convex shaped caracurine V ring system. The absolute configuration of the new stereogenic centers C20 and C20′was determined by a 600 MHz NOESY experiment (see below). An NOE between H-14b (H-14b′), which has an axial orientation in the piperidine ring, and H-20 (H-20′), revealed the axial position of H-20 (H-20′) in the piperidine ring corresponding to an (*S*)-configuration of C-20 and C-20′.



Scheme 1. (a) H_2 (35 bar)/PtO_2, EtOH, 16 h

Conformational Analysis

With the aim of comparing the geometries of caracurine V, *iso*-caracurine V, tetrahydrocaracurine V, and bisnortoxiferine ring systems, their 3D structures were generated and subsequently optimized by semiempirical calculations (AM1) using PC SPARTAN 1.1[12] (Figure 2).

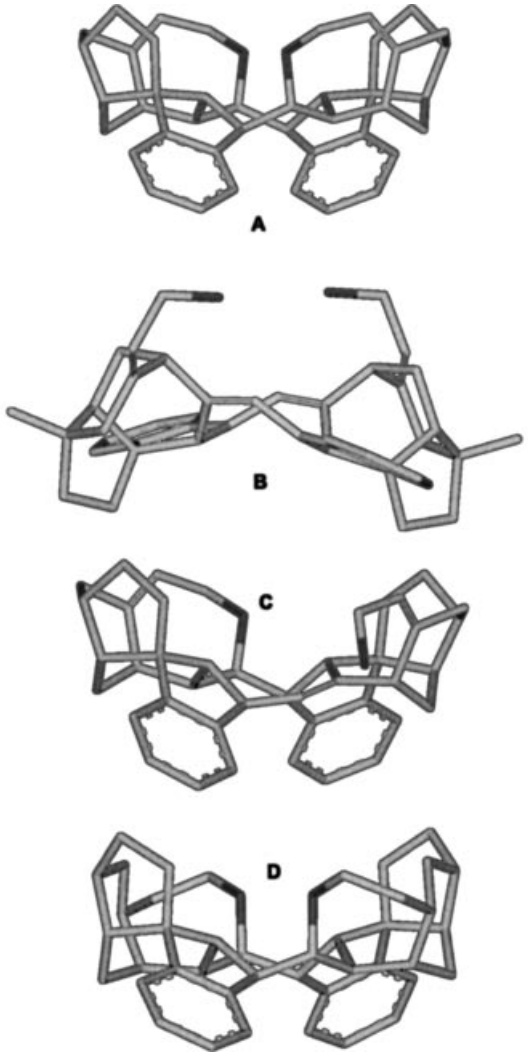


Figure 2. 3D Structures of caracurine V (**4**) (**A**), toxiferine I (**2**) (**B**), *iso*-caracurine V (**5**) (**C**), and tetrahydrocaracurine V (**6**) (**D**)

Caracurine V Ring System

Caracurine V (**4**) was built based on the X-ray structure of strychnine which is the starting compound for its synthesis. The relative configuration within the central diazocane ring has previously been determined by NMR spectroscopy and semiempirical AM1 calculations.[8] Characteristic coupling constants between H-2 and H-16 (J = 10.3 Hz) and H-16 and H-17 (J = 1.8 Hz), as well as a strong NOE between H-17 and H-16 revealed the *cis*-orientation of H-16 and H-17 and the *trans*-orientation of H-2 and H-16. Considering the strychnine-derived axial orientation of H-2 in the diazocane ring, the above-mentioned coupling constants indicate the axial orientation of H-16 and the equatorial position of H-17 in the central ring. Consequently, both oxygen atoms at C-17 and C-17′are likely to adopt axial orientations. The 3D structure of the whole molecule previously derived from 300 MHz NOE-difference spectra[8] was now confirmed by a 400 MHz NOESY experiment (see Supporting Information). The central diazocane ring exists in a crown conformation. The axial position of both oxygen atoms is in agreement with the orientation of the corresponding oxygen atoms found in the X-ray structure of the related caracurine II ring system.[13] The piperidine rings of **4** adopt, as in strychnine, a boat conformation as indicated by a diaxial NOE between protons H-21b and H-14b. The geometry of **4** is displayed in Figure 2 (see A), and the essential NOEs of **4** in $CDCl_3$ solution are shown in Figure 3.

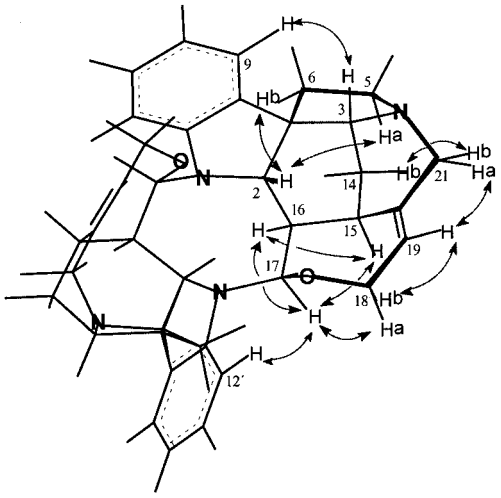


Figure 3. Essential NOEs of caracurine V (**4**; 400 MHz, $CDCl_3$)

Bisnortoxiferine Ring System

Since bisnortoxiferine is a product of a double intramolecular alcohol elimination from the central eight-membered ring of caracurine V (**4**), the 3D structure of the bisnortoxiferine ring system was constructed from the structure of **4** by opening of both lateral tetrahydrooxepine rings, to give free allylic alcohol functions, and two double bonds in the central eight-membered ring. The AM1 calculation revealed a considerable geometrical change of the resulting ring skeleton. While both indole nitrogen atoms of **4** adopt a pyramidal geometry (dihedral angle C12−C13−N−C17′ = −53°), their arrangement within the bisnortoxiferine ring system is flatter (dihedral angle C12−C13−N−C17′ = 21°) due to a conjugation of the nitrogen lone-pairs with the central double bonds. Consequently, the conformation of the central eight-membered ring changed from crown in caracurine V to boat in the bisnortoxiferine ring system. Moreover, opening of the tetrahydrooxepine rings of **4** induced a conformational change in the piperidine rings. While the piperidine rings of **4** adopt boat conformations, their conformations in the less rigid bisnortoxiferine ring system changed to chair as indicated by a 600 MHz ROESY spectrum of toxiferine I (**2**) (see Supporting Information). A ROE between the axial proton H-21b and H-6b and the absence of ROE interactions between any of the hydrogen atoms H-21 and H-14 are only consistent with chair conformations of both piperidine rings. The different conformations of the piperidine rings in **2** and **4** could be confirmed by the vicinal coupling constants between H-14b and H-15. The dihedral angle between H-14b and H-15 in caracurine V (48°) is smaller than for toxiferine I (59°). Consequently, according to the Karplus equation, $J_{14b,15}$ in caracarine V (4.0 Hz) is larger than for toxiferine I (3.3 Hz).The geometry of toxiferine I (**2**) is displayed in Figure 2 (see B), and the essential ROEs of **2** in a DMSO solution are shown in Figure 4.

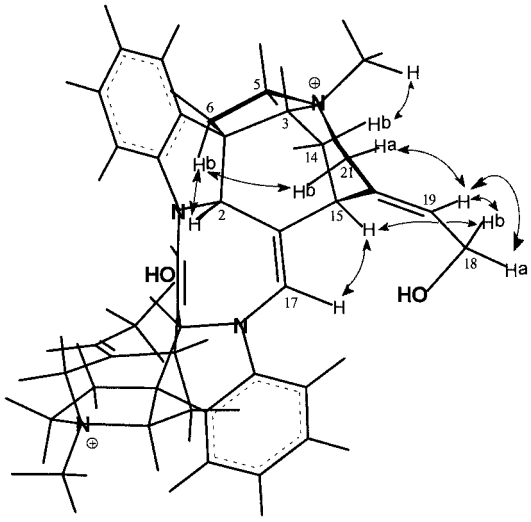


Figure 4. Essential ROEs of toxiferine I (**2**; 600 MHz, $[D_6]$DMSO)

iso-Caracurine V Ring System

iso-Caracurine V (**5**) is built from caracurine V (**4**) in a one-sided intramolecular alcohol elimination from the central diazocane ring. Ring opening at both sides of caracurine V resulting in the bisnortoxiferine ring skeleton led to the conformational change of the central diazocane ring (from crown to chair) as well as of both piperidine rings

(from boat to chair). However, a 600 MHz NOESY spectrum of **5** indicated that opening of only one tetrahydrooxepine ring of **4** did not affect the geometry of the whole ring system very much (applying the ROESY technique led to the appearance of TOCSY cross peaks). Strong NOEs between H-21b and H-14b and H-21b′ and H-14b, as well as similar coupling constants ($J_{14b,15}$ = 4.2 Hz and $J_{14b',15'}$ = 3.9 Hz) revealed the unchanged boat conformation of both piperidine rings. An NOE between H-2 and H-2′ confirmed the concavo-convex shape of the *iso*-caracurine V ring system. The geometry of **5** is displayed in Figure 2 (see C). The essential NOEs of **5** are depicted in Figure 5; the NOESY spectrum of **5** can be found in the Supporting Information.

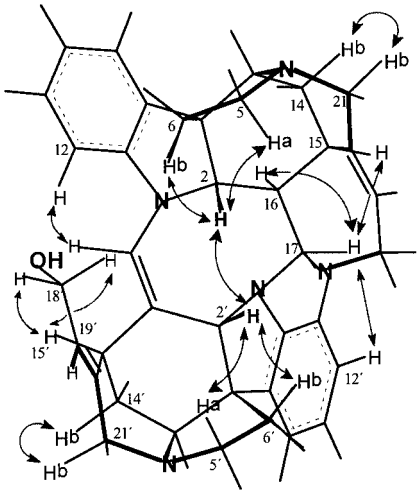


Figure 5. Essential NOEs of *iso*-caracurine V (**5**; 600 MHz, $CDCl_3$)

Tetrahydrocaracurine V Ring System

The 3D structure of tetrahydrocaracurine V (**6**), determined from the 600 MHz NOESY spectrum and subsequent AM1 calculations, revealed a similar relative position of the aromatic rings to those observed for **1** and **4** (as above, applying the ROESY technique led to the appearance of TOCSY cross peaks). However, unlike in **1** and **4**, the piperidine rings of **6** adopt chair conformations, as indicated by NOEs between H-18b and H-21a and H-6b and H-21a, as well as by the absence of NOEs between any of the hydrogen atoms H-21 and H-14. This is in agreement with the geometry of the corresponding piperidine rings found in the X-ray and solution structure of dihydrostrychnine.[14,15] An AM1 calculation for the tetrahydrocaracurine V stereoisomer with boat conformations of the piperidine rings gave a heat of formation 5 kcal/mol higher than for the postulated one. The geometry of **6** is shown in Figure 2 (see D). The essential NOEs of **6** are depicted in Figure 6; the NOESY spectrum of **6** can be found in the Supporting Information.

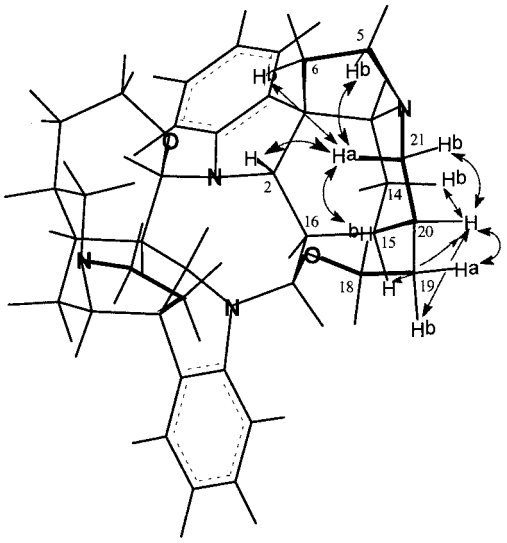


Figure 6. Essential NOEs of tetrahydrocaracurine V (**6**; 600 MHz, $CDCl_3$)

Comparison of the Ring Systems

The spatial arrangement of the pharmacophore consisting of two positively charged nitrogens and two aromatic rings is essential for the receptor−ligand interactions. In order to compare the relative position of the aromatic indole rings in the different ring systems, the structures of *N*,*N*′-dimethyl-*iso*-caracurine V, *N*,*N*′-dimethyltetrahydrocaracurine V, and toxiferine I were superimposed onto *N*,*N*′-dimethylcaracurine V. As shown in Figure 7, the aromatic rings of dimethyl-*iso*-caracurine V and dimethyltetrahydrocaracurine V adopt very similar relative spatial positions compared to the caracurine V ring system. In contrast, after the overlay of one aromatic ring of toxiferine I and dimethylcaracurine V, the other indole rings occupy completely different regions in space. Despite considerable geometrical difference of the caracurine V and bisnortoxiferine ring systems, the N^+-N^+ distance is equal in both ring skeletons (9.7 Å). Nearly the same intercationic distance (9.6 Å) is given in the *iso*-caracurine V ring system. In contrast, due to the conformational change of the piperidine rings, the N^+-N^+-distance in dimethyltetrahydrocaracurine V is reduced to 8.8 Å.

Conclusion

The 3D structures of caracurine V, *iso*-caracurine V, tetrahydrocaracurine V and toxiferine I were determined by means of NMR spectroscopy and semiempirical calculations. The relative spatial arrangement of the aromatic indole rings is similar in all three "caracurine" ring systems. Opening of both tetrahydrooxepine rings of caracurine V to give the bisnortoxiferine ring skeleton leads to the conformational change of the central eight-membered ring

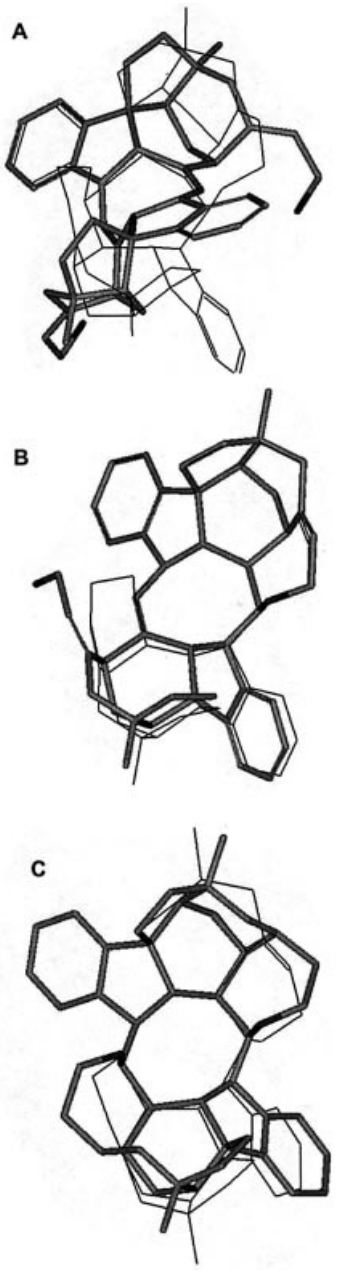


Figure 7. Superposition of toxiferine I (**A**), dimethyl-*iso*-caracurine V (**B**), and dimethyl-tetrahydrocaracurine V (**C**) onto dimethylcaracurine V (line style) (RMS fit of HyperChem. 5.1 ChemPlus Extension, Hypercube, Inc), fitting atoms are aromatic carbon atoms C8−C13

from crown to boat, resulting in a considerably changed geometry of the whole ring skeleton.

Interestingly, the affinity of toxiferine I for the allosteric binding site of muscarinic M_2 receptors is approximately ten times lower than those observed for the other *N*-methyl-substituted compounds, suggesting that the relative orientation of the aromatic rings as given in the "caracurine" ring systems is favourable for an optimal ligand−receptor interaction.[16]

Experimental Section

General: Melting points were determined with a Gallenkamp melting point apparatus (Sanyo) and were not corrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV 400, and Bruker AV 600 instruments. Proton chemical shifts are referred to $CHCl_3$ (δ = 7.24 ppm) and [D_6]DMSO (δ = 2.55 ppm); carbon chemical shifts are referred to $^{13}CDCl_3$ (δ = 77.0 ppm) and [D_6]DMSO (δ = 39.50 ppm). The 600-MHz NOESY spectra of **5** and **6** were recorded with 500-ms mixing times and a recovery delay of 1.8 s. The 400-MHz NOESY spectrum of **4** was recorded with a 800 ms mixing time and a recovery delay of 2 s. The 600 MHz ROESY spectrum of **2** was recorded with a 300 ms mixing time. IR spectra were obtained using a Biorad Pharmalyz IR FT-IR spectrometer. The mass spectrum of **6** was recorded on a Finnigan MAT 8200 spectrometer (70 eV). The mass spectrum of **2** was run by fast-atom bombardment on a Finnigan MAT 90 mass spectrometer in 3-nitrobenzyl alcohol as matrix. Elemental analyses were performed by the microanalytical section of the Institute of Inorganic Chemistry, University of Würzburg. All reactions were carried out under an argon atmosphere.

Toxiferine I (2): This compound was prepared from the dimerization of Wieland−Gumlich aldehyde methochloride (0.45 g) with pivalic acid (10 mL) as described previously by Battersby and Hodson.[11] Yield: 0.23 g (54%). $[\alpha]_D^{22}$ = −651 (*c* = 0.5, DMSO). ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 1.63 (d, *J* = 14.3 Hz, 2 H, H-14a), 2.13 (dt, *J* = 14.3, 3.3, 3.3 Hz, 2 H, H-14b), 2.68 (dd, *J* = 14.5, 8.6 Hz, 2 H, H-6a), 2.80 (m, 1 H, H-6b), 3.24 (s, 6 H, 2 × CH_3), 3.74 (br. s, 2 H, H-3), 3.76−3.87 (m, 6 H, H-15, H-5a, H-21b), 4.04 (m, 2 H, H-5b), 4.14−4.53 (m, 6 H, H-21b, CH_2–18), 5.10 (t, *J* = 5.3 Hz, 2 H, -OH), 5.79 (t, *J* = 6.4 Hz, 2 H, H-19), 5.86 (s, 2 H, H-2), 6.54 (d, *J* = 7.6 Hz, 2 H, H-12), 6.62 (s, 2 H, H-17), 6.90 (td, *J* = 7.6, 7.6, 1.0 Hz, 2 H, H-10), 7.23 (td, *J* = 7.6, 7.6, 1.0 Hz, 2 H, H-11), 7.60 (dd, *J* = 7.6, 1.0 Hz, 2 H, H-9) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 20.8 (C14), 29.2 (C15), 38.2 (C6), 52.4 (C7), 56.6 (C18), 59.5 (C5), 63.8 (C21), 68.2 (C2), 75.1 (C3), 108.1 (C12), 111.8 (C16), 119.9 (C10), 124.4 (C9), 129.8 (C11), 130.5 (C19), 131.2 (C17), 133.7 (C8), 134.1 (C20), 145.2 (C13) ppm. FAB-MS: *m*/*z* = 651.2 and 649.2 [$M^{2+}Cl^-$], 613.3 [M^{2+} − H^+)], 595.3.

(20*S*,20′*S*)-19,20,19′,20′-Tetrahydrocaracurine V (6): PtO_2 (15 mg) was added to a solution of **6** (50 mg) in ethanol (20 mL). The reaction mixture was hydrogenated under a hydrogen pressure of 35 bar for 16 h. The catalyst was removed by filtration through Celite and the filter pad was washed with ethanol (2 × 5 mL). Evaporation of the solvent afforded NMR-pure colourless crystals of **6** (50 mg, 100%) m.p. > 300 °C (dec.). $[\alpha]_D^{22}$ = −9 (*c* = 0.54, $CHCl_3$). IR (ATR): $\tilde{\nu}$ = 2926, 2879, 1597, 1483, 1454, 1267, 1156, 1077, 747, 689 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.18 (m, 2 H, H-19a), 1.74 (dt, *J* = 13.6, 2.5, 2.5 Hz, 2 H, H-14a), 1.91 (m, 4 H, H-15, 6a), 2.00 (m, 2 H, H-19b), 2.19 (br. d, *J* = 13.6 Hz, 2 H, H-14b), 2.30 (dd, *J* = 8.5, 5.4 Hz, 2 H, H-16), 2.35 (m, 2 H, H-20), 2.64 (dt, *J* = 14.1, 8.6, 8.6 Hz, 2 H, H-6b), 3.02 (m, 4 H, H-5a, 21a), 3.20 (m, 2 H, H-21b), 3.45 (m, 2 H, H-5b), 3.72 (m, 4 H, H-3, 18a), 3.79 (td, *J* = 11.8, 11.8, 7.1 Hz, 2 H, H-18b), 4.51 (d, *J* = 8.5 Hz, 2 H, H-2), 4.73 (br. s, 2 H, H-17), 6.55 (d, *J* = 8.0 Hz, 2 H, H-12), 6.86 (td, *J* = 7.6, 7.6, 0.9 Hz, 2 H, H-10), 6.99 (dd, *J* = 7.6, 0.9 Hz, 2 H, H-9), 7.13 (ddd, *J* = 8.0, 7.6, 0.9 Hz, 2 H, H-11) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.9 (C14), 30.0 (C19), 34.5 (C15), 36.9 (C20), 40.7 (C6), 52.1 (C21), 53.1 (C5), 53.8 (C16), 54.2 (C7), 59.8 (C2), 61.9 (C3), 65.2 (C18), 91.5 (C17), 112.1 (C12), 121.6 (C9), 122.0 (C10), 129.5 (C11), 132.4 (C8), 151.7 (C13) ppm.

MS (EI): *m/z* (%) = 588 (100) $[M]^+$, 297 (24). $C_{38}H_{44}N_4O_2$ (588.8): calcd. C 77.52, H 7.53, N 9.52, found C 77.27, H 7.31, N 9.47.

Acknowledgments

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