Gardenamide A from *Rothmannia urcelliformis* (Rubiaceae) − Isolation, Absolute Stereostructure, and Biomimetic Synthesis from Genipine

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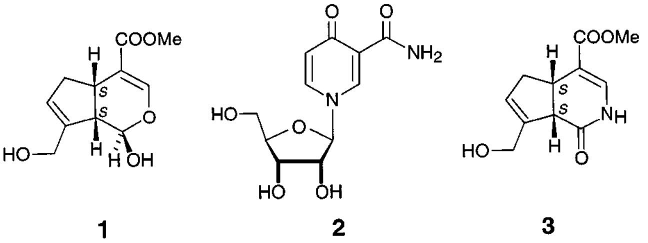
The iridoid alkaloid gardenamide A (**3**) was isolated from the phytochemically uninvestigated East African Rubiaceae species *Rothmannia urcelliformis* (Hiern) Bullock. Its absolute stereostructure was determined by quantum chemical CD calculations, which constitutes the first application of this useful chiroptical tool within the class of iridoids. An additional stereochemical proof succeeded by its biomimetic partial synthesis starting from the well-known nitrogen-free iridoid genipine (**1**).

Introduction

Rothmannia urcelliformis (Hiern) Bullock is a small tree that is widespread in the forests of East Africa.[1] While related *Rothmannia* species are used in traditional medicine for the treatment of diarrhoea and as analgesics, extracts of *R. urcelliformis* itself are employed as an arrow poison.[1] Phytochemically, only three *Rothmannia* species have so far been investigated, giving rise to a variety of very different metabolites. Thus, the South African species *R. globosa* has been found to contain several iridoids, among them the widespread cyclic semiacetal genipine (**1**) (see Figure 1),[2] whereas from *R. longiflora*, the interesting 4-oxonicotinamide **2**,[3] which had previously only been found in mammals,[4] was isolated; no iridoids were found. No secondary metabolites have so far been reported from *R. hispida*. The only compound thus far isolated is a dye which has not yet been fully characterized.[5]



Figue 1. Genipine (**1**), 4-oxonicotinamide-1-(1′-β-D-ribofuranoside) (**2**), and gardenamide A (**3**) isolated from different *Rothmannia* species

This as yet incoherent diversity of structures makes the search for further secondary metabolites of other *Rothmannia* species a rewarding task. In this paper, we report the first phytochemical investigation of *R. urcelliformis* resulting in the isolation of gardenamide A (**3**, see Figure 1) and the elucidation of its previously unknown absolute configuration by quantum chemical circular dichroism (CD) calculations. We also report the confirmation of this stereochemical assignment by the biomimetic synthesis of **3** from **1**.

Results and Discussion

R. urcelliformis was collected in March 1997 near Harare, Zimbabwe. Fractionation of the CH_2Cl_2 extract of the fruits by CC on silica gel and finally by MPLC on RP-18 gave 7 mg of an amorphous solid. The first ^1H NMR investigations, together with the molecular formula $C_{11}H_{13}NO_4$ (from high resolution mass spectrometry) indicated the presence of an iridoid alkaloid.

The low-field-shifted signal of the olefinic proton at C-3 ($\delta = 7.19$, Figure 2a) showed HMBC (Heteronuclear Multiple Bond Correlation) interactions with C-4 ($\delta = 111.2$, Figure 2b) and with a ^{13}C NMR signal with a chemical shift typical of a carboxy carbon atom ($\delta = 168.9$) which displayed an HMBC interaction with a 3-proton signal characteristic of a methyl ester group ($\delta = 3.73$). The HMBC interaction between 3-H and C-1 ($\delta = 172.8$), together with the interaction between C-1 and a signal at $\delta = 3.59$ permitted an assignment of the latter as 9-H. 5-H ($\delta = 3.52$) was identified by its HMBC interactions with C-3, C-4, and C-9. The spin-system pattern of 5-H and of the two diastereotopic protons at C-6 ($\delta = 2.16$ and 2.86) allowed the identification of CH_2-6 and CH-7 ($\delta = 5.84$), which was confirmed by the above-mentioned HMBC and further H,H-COSY experiments (see Figure 2b). The HMBC interactions of the two (nearly isochronic) CH_2 protons of the hydroxymethyl group ($\delta = 4.36$ and 4.38) and of 7-H and

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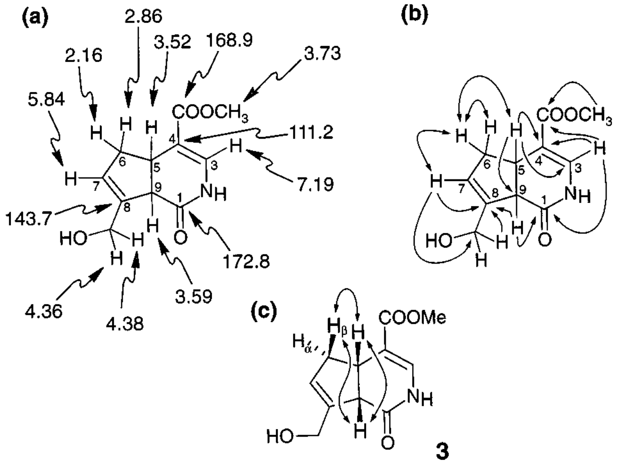
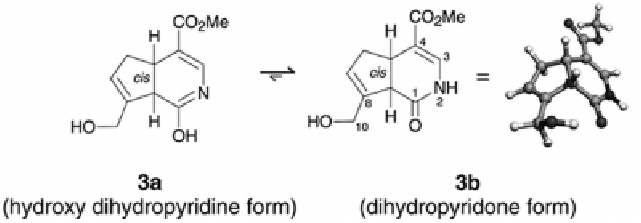


Figure 2. Selected ^1H and ^{13}C NMR shifts (δ values in ppm) (a), HMBC (single arrows) and H,H-COSY interactions (double arrows) (b) relevant for the constitution; relative configuration at the stereogenic centers of gardenamide A (**3**) through ROESY interactions (c)

9-H with C-8 (δ = 143.7) allowed location of the CH_2OH group at C-8. The relative stereoarray at CH-5 and CH-9 of the resulting structure **3** was deduced to be *cis* by their ROESY (Rotating Frame Overhauser Enhancement Spectroscopy) interactions (Figure 2c).

A compound with the same make-up and optical rotation, named gardenamide A (or sometimes just gardenamide[6]), had previously been isolated from *Gardenia jasminoides*.[6] The authors of that paper had postulated the absolute configuration of the alkaloid to be (5*S*,9*S*), yet without giving any evidence of this stereochemical assignment. Even if this configuration appears to be relatively probable since it is more frequently found within this class of compounds,[7] a couple of iridoids have been identified with other absolute (and relative) configurations.[8] Absolute configurations of such iridoids were elucidated by comparison of the CD spectra with those of related compounds whose absolute configurations were known e.g. through X-ray structure analyses[9−12] in only a few cases. For the unambiguous assignment of the absolute stereostructure of gardenamide A from *R. urcelliformis*, we have now used the method of quantum chemically calculating CD spectra and comparing them with the experimental ones.[13−15] This constitutes the first application of this useful and reliable method in the class of iridoid alkaloids − and in iridoids at all.

Semiempirical AM1 calculations in the gas phase showed that the imaginable tautomeric hydroxydihydropyridine form **3a** does not have to be taken into account for the CD calculations since it is by approximately 12 kcal/mol less stable than the dihydropyridone form **3b** (Scheme 1). The predominance of **3b** in solution is also in agreement with the chemical shift of the C-1 signal of δ = 172.8. Furthermore, **3b** has a significantly higher dipole moment (3.88 D) than **3a** (1.88 D). Therefore this tautomer should be further stabilized by the solvent.

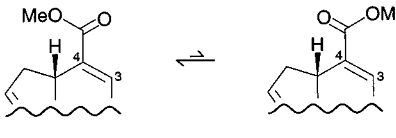


3a
(hydroxy dihydropyridine form)

3b
(dihydropyridone form)

Scheme 1. The two possible tautomeric forms **3a** and **3b** of gardenamide A; calculated minimum structure of **3b** [here for the (*S*,*S*) enantiomer]

Arbitrarily starting with the (*S*,*S*)-configured enantiomer of **3b**, the semiempirical conformational analysis (AM1) preceding the CD calculations revealed the presence of only two flexible parts in the molecule, due to the rigid framework of gardenamide A. The ester substituent at C-4 lies in the plane of the unsaturated lactam ring, thus permitting conjugation of the carbonyl group with the electron-rich double bond between C-3 and C-4, and therefore shows two stable rotameric arrays (see Scheme 2). The conformer in which the carbonyl oxygen atom of the ester group is oriented to the "right" side, i.e. towards this double bond, and the methoxy group towards the 5-memberd ring, is energetically slightly (by ca. 0.10−0.15 kcal/mol) preferred compared to the conformational array in which the methoxy group is oriented towards the endocyclic double bond.



Scheme 2. The two stable conformational orientations of the ester substituent of gardenamide A (**3**)

The rotation of the hydroxy function at C-10 around the C-10−C-8 bond leads to five stable conformational arrays. In two of these cases, the hydroxy substituent forms a hydrogen bond, albeit relatively weak (d_{H-O} = 215−220 pm), to the carbonyl group at C-1.

The degrees of conformational freedom of these ester and hydroxymethyl substituents are independent of each other, thus leading to 2 × 5 = 10 conformers, whose heats of formation lie within a small range of only 2.20 kcal/mol. For this reason, all of these conformers are energetically relevant and were thus considered for the calculation of the chiroptical properties of gardenamide A (**3**). The computed single CD spectra of each minimum structure were Boltzmann-weighted according to the heat of formation of the corresponding conformer, and "UV-corrected"[13,14] (see Figure 4) to give the calculated overall CD spectrum for (*S*,*S*)-**3**. By reflection of this curve at the zero line, the predicted spectrum was obtained for (*R*,*R*)-**3**, too. The comparison of the two computed spectra with the experimental one of gardenamide A (**3**) shows a very good agreement for the spectrum calculated for (*S*,*S*)-**3** (see Figure 3, left spectrum), whereas the theoretical one for (*R*,*R*)-**3** is nearly the mirror image of the experimental spectrum (see Figure 3, right spectrum). With this result, the absolute configuration of gardenamide A can unambiguously be assigned as (*S*,*S*).

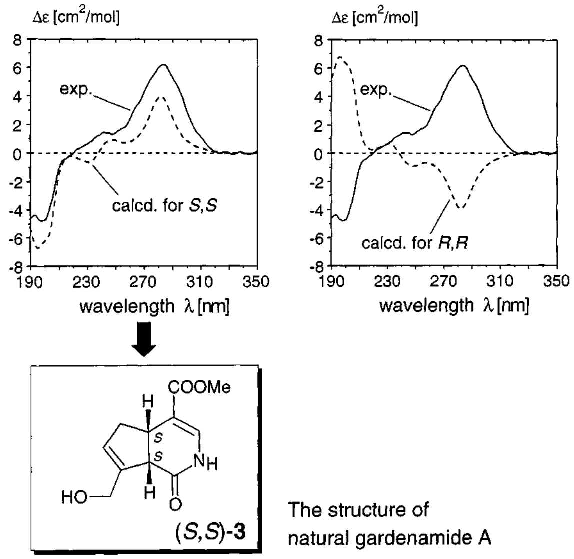
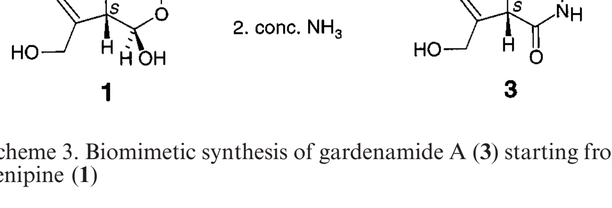


Figure 3. Attribution of the absolute configuration of gardenamide A (**3**) by comparison of the spectra calculated for (*S*,*S*)- and (*R*,*R*)-**3** with the experimental one (in EtOH)

Iridoids constitute an important class of natural products; moreover, they are biosynthetic precursors to a broad range of indol alkaloids, with joint biosynthetic key intermediates like loganin and its oxidative cleavage product, secologanin.[16] Given the in some cases well-investigated incorporation of nitrogen atoms into iridoids (e.g. via amino acids) to give iridoid alkaloids,[16−18] a − presumably biomimetic − partial synthesis of gardenamide A (**3**) from its nitrogen-free putative precursor genipine (**1**) seemed rewarding, also for a further proof of the absolute configuration of **3**. The absolute configuration of genipine (**1**) itself had previously been assigned in the course of a partial synthesis of the related natural product udoteatrial hydrate from **1**.[19]

Genipine (**1**), which occurs in a variety of plant species,[2,20−22] was presented in previous isolation work on *Pavetta lanceolata*.[23] The conversion was achieved by oxidation of the semiacetal functionality of **1** with Ag^I carbonate, using conditions similar to those previously used for the oxidation of nepetalactol,[24] followed by the incorporation of a nitrogen atom using ammonia (Scheme 3). The product obtained thus proved to be fully identical to gardenamide (**3**), in all its physical and spectroscopic data, including the chiroptical properties, thus fully confirming the absolute configuration of **3** as assigned above.



Scheme 3. Biomimetic synthesis of gardenamide A (**3**) starting from genipine (**1**)

Conclusion

This work constitutes the first assignment of the absolute stereostructure of gardenamide A (**3**), by quantum chemical CD calculations, illustrating the usefulness of this method, in the field of iridoids for the first time. The partial synthesis of **3** starting from the configurationally known non-alkaloidal precursor genipine (**1**) in a one-pot procedure, by oxidation and incorporation of ammonia, confirms the structural assignments and underlines the reliability of the CD calculations.

Experimental Section

General Remarks: Melting points were determined with a Reichert-Jung Thermovar hot-plate and are uncorrected. − Optical rotations were taken with a Perkin−Elmer 241MC polarimeter (25 °C, 10-cm cell). − IR spectra with a Perkin−Elmer 1429 spectrophotometer. − CD spectra (25°C, EtOH, 0.1-cm cell) with a Jasco J-715 spectropolarimeter. − ^{1}H NMR (600.13 MHz) and ^{13}C NMR (150.9 MHz) spectra were measured with a Bruker DMX 600 instrument using CD_3OD (δ = 3.30 and 49.0) as the solvent and internal ^{1}H and ^{13}C standard. Proton-detected, heteronuclear correlations were measured using HMQC (Heteronuclear Multiple Quantum Correlation, optimized for $^1J_{HC}$ = 145 Hz) and HMBC (optimized for $^nJ_{HC}$ = 7.0 Hz). EIMS and HRMS were determined with Finnigan MAT 8200 and Finnigan MAT 90 instruments (70 eV). − For TLC, precoated silica gel 60 F_{254} plates (Merck, 5 × 10 cm) were used. Spots were detected under UV light. − Column chromatography was carried out on silica gel 60 (60−200 mesh, Merck). For MPLC a Lobar (Merck) Li Chroprep RP-18 (40−63 μm) column (310 × 25 mm) was used with a flow of 9 mL min^{-1} and UV detection at 254 nm, solvent: H_2O/acetonitrile (88:12). Organic solvents were dried and distilled prior to use.

Plant Material: The plant material was collected by Stephen Duri near Harare, Zimbabwe, in March 1997 and identified by Dr. Jan Schlauer.

Isolation of Gardenamide A (3): Dried fruits of *R. urcelliformis* (400 g) were powdered and extracted successively with petroleum ether and CH_2Cl_2 to yield 7.00 g of a petroleum ether extract and 7.69 g of a brownish crude CH_2Cl_2 extract, the latter of which was chromatographed on silica gel using CH_2Cl_2/MeOH (95:5 up to 85:15) as the eluent. Further purification on RP-18 MPLC gave **3** (7.0 mg) as an amorphous solid. − $[\alpha]_D^{20}$ = +402.3 (*c* = 0.16, MeOH) [ref.[6] +404.0 (*c* = 0.25, MeOH)]. − CD: $\Delta\varepsilon_{198}$ = −3.71, $\Delta\varepsilon_{284}$ = 4.73 (*c* = 0.04, EtOH). − IR (NaCl): $\tilde{\nu}$ = 3200, 2920, 1670, 1640, 1430, 1290, 1220, 1100, 760 cm^{-1}. − ^{1}H NMR (600 MHz, CD_3OD): δ = 2.16 (dddt, 1 H, *J* = 16.4, 8.8, 2.2, 2.2 Hz, 6-H$_\alpha$), 2.86 (dddt, 1 H, *J* = 16.4, 8.4, 2.8, 1.4 Hz, 6-H$_\beta$), 3.52 (dt, 1 H, *J* = 10.7, 8.7 Hz, 5-H), 3.59 (m, 1 H, 9-H), 3.73 (s, 3 H, $COOCH_3$), 4.36 (dd, 1 H, *J* = 14.1, 1.7 Hz, 8-CHOH), 4.38 (dd, 1 H, *J* = 14.1, 1.2 Hz, 8-CH′OH), 5.84 (m, 1 H, 7-H), 7.19 (s, 1 H, 3-H). − ^{13}C NMR (150 MHz, CD_3OD): δ = 38.8 (CH-5), 41.0 (CH_2-6), 49.4 (CH-9), 52.0 (COO*C*H_3), 61.8 (CH_2OH), 111.2 (C-4), 128.1 (CH-7), 134.9 (CH-3), 143.7 (C-8), 168.9 (*C*OOCH_3), 172.8 (C-1). The ^{13}C assignments were achieved by HMQC and HMBC experiments. − MS (EI, 70 eV); *m/z* (%): 223 $[M]^+$ (39), 208 $[M - CH_3]^+$ (12), 205 $[M - H_2O]^+$ (23), 192 $[M - CH_2OH]^+$ (11), 164 $[M - COOMe]^+$ (11), 154 (100). − HRMS: 223.08428 ($C_{11}H_{13}NO_4$ calcd. 223.08446).

Synthesis of 3: Genipine (2.0 mg, 8.8 μmol) (present from previous isolation work on *Pavetta lanceolata*[23]) was refluxed for 2 h in dry benzene (2.0 mL) with Ag_2CO_3 (4.0 mg, 14.5 μmol) and Celite (5.0 mg). Conc. aqueous NH_3 solution (0.5 mL) was added, the solvent was removed, and the residue purified by prep. TLC to yield 1.4 mg (71.3%) of **3**. − $[\alpha]_D^{20}$ = +401.8 (*c* = 0.09, MeOH). − Identical with the isolated sample by TLC, HPLC coelution (μBondapak, detection at 254 nm, flow rate: 1 mL/min, solvent: 0:100 up to 20:80 in 30 min), ^{1}H NMR and CD.

Conformational Analyses: The conformational analysis of gardenamide A (**3**) was performed with Silicon Graphics OCTANE R10000 workstations by means of the AM1[25] parameterization as implemented in the program package VAMP 6.5,[26] starting from preoptimized geometries generated by the TRIPOS[27] force field.

CD Calculations: The wavefunctions required for the calculation of the rotational strengths for the electronic transitions from the ground state to excited states were obtained by CNDO/*S*-CI calculations[28] with a CI expansion including 576 singly occupied configurations and the ground state determinant. These calculations were carried out with LinuX PentiumII workstations by the use of the BDZDO/MCDSPD[29] program package. All single CD spectra obtained in this way were added up by the Boltzmann statistics using appropriate heats of formation, to give the calculated overall CD spectrum. For a better visualization, the rotational strengths were transformed into $\Delta\varepsilon$ values and superimposed with a Gaussian band shape function. The assignment of responsible transitions of the relevant calculated CD bands at 195 and 282 nm was done by the examination of the involved molecular orbitals and the comparison of the experimental and calculated UV spectra (which is shifted slightly to lower wavelengths), which agree nearly perfectly (see Figure 4). In accordance with this small deviation, the calculated CD spectrum was corrected by shifting it by 10 nm to higher wavelengths, for better comparison. For further details concerning the quantum chemical CD calculations, of chiroptical properties see the corresponding section in ref.[13]

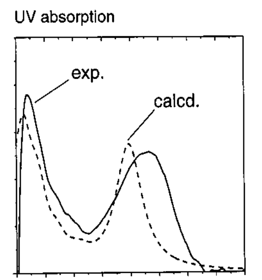


Figure 4. Comparison of the experimental UV spectrum of gardenamide A (**3**) with the calculated one

Acknowledgments

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