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Absolute configuration of indoline alkaloids from *Geissospermum reticulatum*

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ABSTRACT

The absolute configuration (AC) of (+)-2*R*,7*R*,15*R*,17*S*,19*S*-10-demethoxy-12-hydroxy-17,19-epoxygeissovelline (**1**), a main aspidospermatan-type indoline alkaloid from *Geissospermum reticulatum*, was established by vibrational circular dichroism (VCD), facilitating deduction of the AC of all structurally related alkaloids isolated from this plant.

1. Introduction

Geissospermum reticulatum (Apocynaceae) is a tree commonly found throughout the Amazon rain forest of South America.[1] Species of this genus are a rich source of indole monoterpene alkaloids possessing strychnan, corynanthean, aspidospermatan, or flavopereirine β-carboline type skeletons.[2] There are several reports addressing indole alkaloids' wide-spread biological activity, biosynthetic-pathway studies, and total synthesis.[3–8] In addition, a recent review[9] on the absolute configuration determination of monoterpene indole alkaloids using electronic circular dichroism has been published but without consideration of aspidospermatan type alkaloids.

Recently, the structural elucidation of the aspidospermatan-type alkaloids **1**–**3** derived from geissosvelline, and the newly defined geissospermidine-subtype alkaloids **4**–**7**, found as constituents of *G. reticulatum* leaves and bark (Scheme 1), was described.[10] From a stereochemical viewpoint, the spiro indoline and cycloazanonanone rings tethering by the C-14 methylene group, in alkaloids **1**–**3**, form a complex shaped molecule which resembles a butterfly, where the C-7 spiro- and the C-15 chiral centers are the head and the tail, respectively. The relative stereochemistry of the C-2, C-7, and C-15 chiral atoms of the aspidospermatan skeleton is determined by this capricious shape.

Alkaloids **1**–**7** are newly reported monoterpenes,[10] and a better understanding of their biogenetic pathways prompted us to determine the AC of (+)-10-demethoxy-12-hydroxy-17,19-epoxygeissovelline (**1**), the essential component of *G. reticulatum*. Determination of this molecule configuration would make possible AC deductions for aspidospermatan alkaloids **2**–**7** as well as others isolated from different species of the same genus. Furthermore, AC determination of aspidospermatan-type alkaloids is relevant to the asymmetric synthesis of related indoline alkaloid scaffolds,[11] structure–activity relationships for drug screening,[12] and tumor cell cytotoxicity.[13] Thus, the AC of (+)-**1** was scrutinized by VCD, a chiroptical technique with a growing reputation in the natural product field for absolute configuration assignments.[14–17]

2. Results and discussion

A successful spectroscopic prediction of the absolute configuration of a molecule must be done using a model which provides molecular connectivity and relative stereochemistry with accuracy. Thus, the AC assignment of **1** was appointed after establishment of its connectivity and relative stereochemistry by NMR spectroscopy and X-ray diffraction.[10] Moreover, the X-ray diffraction analysis of **1** was repeated here to ride structural parameters quality high [$R = 3.1\%$ (this work) vs $R = 5.3\%$ (Ref. 10)], achievement made by the use of a scintillation counter equipped diffractometer which provides a higher accuracy in data collection than the CCD diffractometer employed previously.[10] The updated crystal structure[18] is provided in Figure 1, wherein it can be seen the acetyl methyl group is disordered[19] as was the case in several

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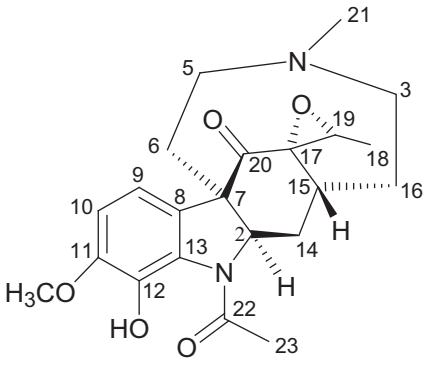
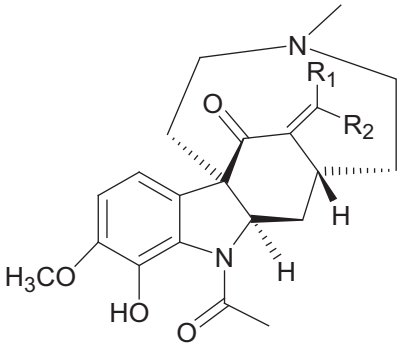
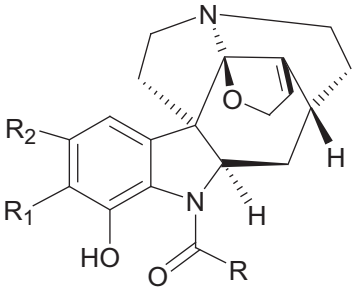


1



2, $R_1 = CH_3$; $R_2 = H$
3, $R_1 = H$; $R_2 = CH_3$



4, $R = CH_3$; $R_1 = R_2 = H$
5, $R = CH_3$; $R_1 = H$; $R_2 = OCH_3$
6, $R = CH_2CH_2CH_3$; $R_1 = R_2 = H$
7, $R = CH_3$; $R_1 = OCH_3$; $R_2 = H$

Scheme 1. Structures for indoline alkaloids **1**–**7**. The atom numbering derives from biogenetic reasons (see text).

N-methyloxindoles.[20] It is worth mentioning that we were unable to determine the AC of **1** by X-ray diffraction, even though in very favorable cases it is possible to do so for molecules containing oxygen as the heaviest atom. Thence, the structure in Figure 1 confirms the relative stereochemistry of the C2, C7, C15, C17, and C19 chiral centers. The atom co-ordinates derived from the X-ray analysis were used as the starting point for the conformational analysis required by VCD.

Infrared (IR) absorption and VCD measured spectra[21] are shown in comparison with their calculated spectra afterward. Compound **1**, $C_{22}H_{28}N_2O_5$, has a total of 57 atoms, 214 electrons, and 165 degrees of freedom. The atom numbering used in this work derives from the biogenetic origin of monoterpene indoline alkaloids condyfoline, condylocarpine, and tubotaiwine.[22–24]

VCD spectral simulations of 2*R*,7*R*,15*R*,17*S*,19*S*-**1** were started by seeking for those low-lying energy conformers that sketch its rotameric landscape. The atom co-ordinates of the crystal structure of **1** were imported to the Spartan '04 software platform employed for the conformational search. The results of the molecular mechanics force field (MMFF94) calculations provided 20 conformers in the first 10 kcal mol^{-1}, which under single point energy calculations with DFT at the B3LYP/6-31G(d) level of theory shorten the landscape to five conformers cornered to the first 2.2 kcal mol^{-1} (Table 1).

Geometry optimization of the five conformers at the B3LYP/DGDZVP level of theory, in GAUSSIAN'03, redistributed energy values placing one of the conformers under 1% of the Boltzmann population, a negligible contribution to conformational equilibria, therefore allowing reduction to four conformers (Fig. 2) for further vibrational spectra simulations. Conformers **a** and **b** are considerably more populated (92.4%) than conformers **c** and **d** (7.6%). The difference in conformation between conformers **a** and **b** is in the rotameric methoxy group at C-11. There is a hydrogen bond between the hydroxy group at C-12 and the carbonyl of the *N*-acetyl group in conformers **a**–**c** which is no longer occurring in conformer **d**. None of the conformers have the same geometry than the crystal structure although conformer **b** has resemblances in all but the rotameric conformation of the methoxy methyl group at the indoline moiety. This small difference is likely due to crystal packing.

Harmonic vibrational frequencies and intensities calculated at the B3LYP/DGDZVP level of theory for the four selected conformers were converted into IR and VCD spectra using Lorentzian line shapes with a bandwidth of 6 cm^{-1}. The visual comparison of experimental and calculated free energy averaged IR and VCD spectra at room temperature, in the fingerprint region, is presented in Figure 3. Of relevance is an intense band closely reproduced in frequency and shape between the experimental (b) and calculated (a) IR spectra which arises from the C–O stretching mode occurring at 1250 cm^{-1} in spectrum (b) and at 1274 cm^{-1} in spectrum (a).

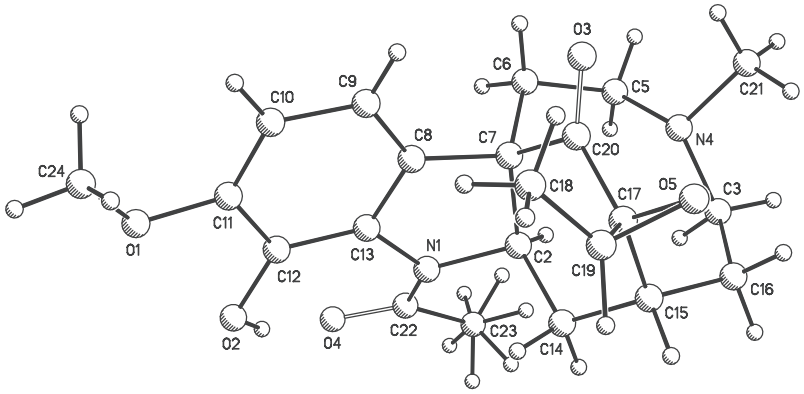


Figure 1. Crystal structure of (+)-10-demethoxy-12-hydroxy-17,19-epoxygeissovelline (**1**). The acetyl methyl group is disordered.[19,20]

Table 1
Relative energies and conformational populations (%) of **1**

Conformer	ΔE_{MMFF94}[a] (kcal mol^{-1})	$\%_{MMFF94}$	ΔE_{SP}[a] (kcal mol^{-1})	$\%_{SP}$	$\Delta\Delta G_{OPT}$[a] (kcal mol^{-1})	$\%_{OPT}$
a	0.78	12.5	1.88	3.2	0.0[d]	77.2
b	2.81	0.4	0.0[c]	75.8	0.96	15.2
c	4.83	0.0	2.17	1.9	1.75	4.0
d	0.08	40.6	1.08	12.2	1.81	3.6
e	0.0[b]	46.5	1.42	6.9	–	–

[a] Relative to the lowest energy conformer.
[b] E_{MMFF} = 91.63 kcal/mol.
[c] DFT ΔE_{SP} = −841,337.10 kcal/mol.
[d] DFT ΔG_{OPT} = −841,179.69 kcal/mol.

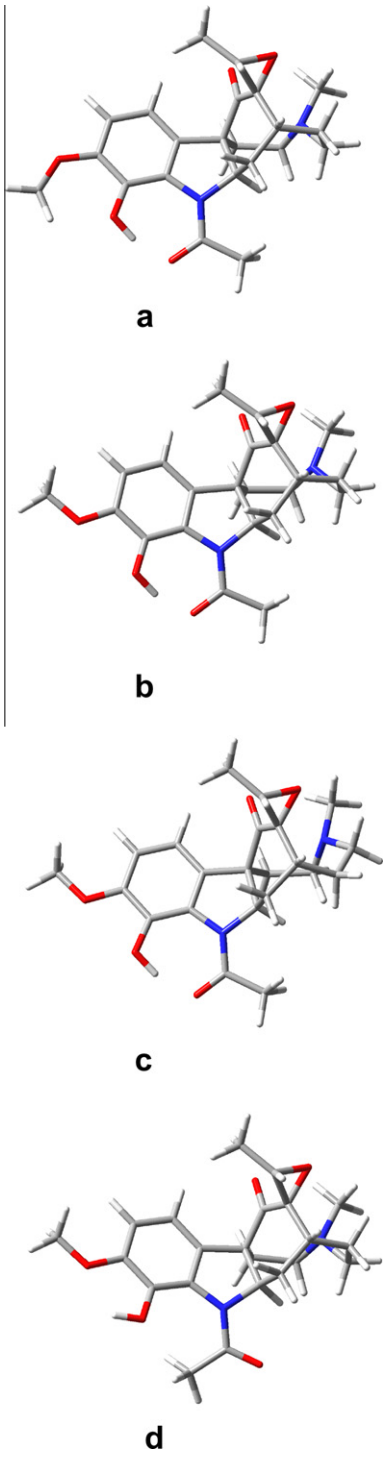


Figure 2. Lowest-lying conformers of 2*R*,7*R*,15*R*,17*S*,19*S*-epoxygeissovelline **1**.

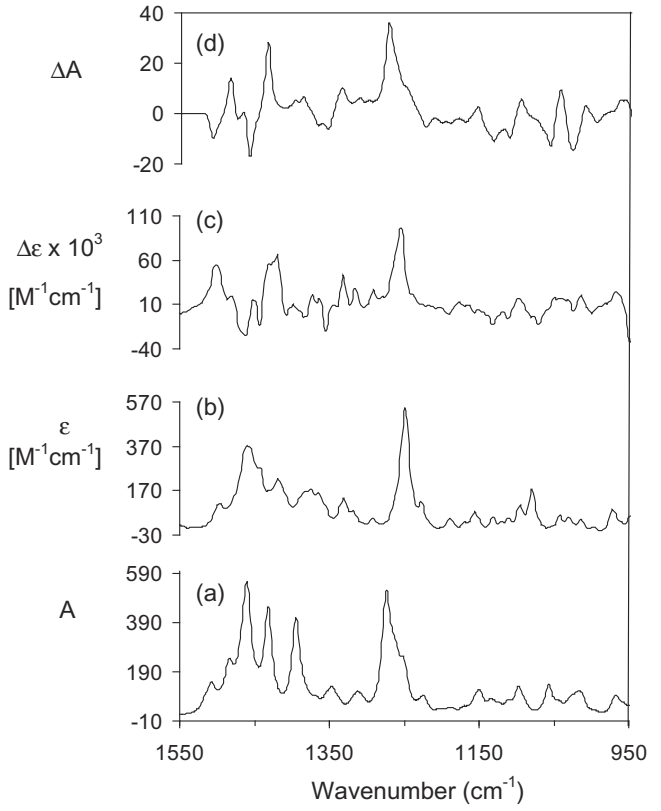


Figure 3. Vibrational spectra of (+)-(2*R*,7*R*,15*R*,17*S*,19*S*)-10-demethoxy-12-hydroxy-17,19-epoxygeissovelline (**1**). (a) Calculated IR using an*H* = 0.981, (b) experimental IR in $CDCl_3$, (c) experimental VCD in $CDCl_3$, (d) calculated VCD using an*H* = 0.981.

Likewise, correlation involving the C–O stretching band in experimental (c) and calculated (d) VCD spectra is similar in frequency and of positive sign at 1256 cm^{-1} and at 1273 cm^{-1}, respectively. In overall balance, VCD spectra comparison favors similarities more than differences indicating that the arbitrarily drawn 2*R*,7*R*,15*R*,17*S*,19*S* absolute configuration in the crystal structure of the (+)-**1** enantiomer is truly correct. ACs of structurally related alkaloids **2**–**7**, isolated from *G. reticulatum*, follow deduction from their reported molecular connectivity and relative stereochemistry,[10] along with the skeleton chiral centers 2*R*,7*R*,15*R* assignments for **1**, since according to plant biogenesis all derivatives should follow a common natural biosynthetic pathway.

Seeking to gain precision in the absolute configuration determination of (+)-**1**, neighborhood similarities of experimental and simulated IR and VCD spectra were calculated with the Compare*VOA* program.[25] Indeed, IR spectra similarity index (S_{IR}) of 87.4 and VCD spectra similarities of 71.7 for the correct enantiomer (S_E) and of 22.2 for the antipode (S_{-E}) that lead to a difference *ESI* value

of 49.5 confirmed (+)-2*R*,7*R*,15*R*,17*S*,19*S* assignment for indoline alkaloid **1** with 100% confidence.

3. Conclusion

In summary, the AC determination of (+)-2*R*,7*R*,15*R*,17*S*,19*S*-10-demethoxy-12-hydroxy-17,19-epoxygeissovelline (**1**) was assessed by VCD spectroscopy. The handedness of aspidospermatan-type alkaloids **2**–**7** was deduced from their connectivity, relative stereochemistry, and the AC determination of skeleton chiral centers 2*R*,7*R*,15*R* of **1**. The two most populated conformers **1a** and **1b** have a difference in the indoline methoxy group although the butterfly-shaped skeleton remains the same. The crystal structure of **1** shows disorder. The C–O stretching (1250–1274 cm^{-1}) is a marker band which facilitates VCD experimental to calculated visual spectra comparison. The AC assignment was quantitatively confirmed with 100% confidence.

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18. The crystal data for **1**, mp 316–318 °C, $C_{22}H_{28}N_2O_5$, M = 400.46, were collected on a Bruker–Nonius CAD4 diffractometer equipped with CuKα radiation (λ = 1.54184 Å) at 293(2) K in the ω-2θ scan mode. Unit cell refinements using 25 machine centered reflections were done using the CAD4 Express v2.0 software. The crystal was orthorhombic, space group $P2_12_12_1$, a = 11.520(2) Å, b = 13.012(1) Å, c = 13.371(2) Å, V = 2004.3(5) $Å^3$, Z = 4, ρ = 1.33 mg/mm^3, μ(CuKα) = 0.771 mm^{-1}, total reflections = 1455, unique reflections = 1362 (R_{int} 0.01%), observed reflections = 1325, final R indices [$I > 2\sigma(I)$] R_f = 3.1%, R_w = 8.6%. The structure was solved by direct methods using the SHELXS-97 program included in the WinGX v1.70.01 crystallographic software package. The highest residual peak in the final difference Fourier map showed an electron density of 0.105 e/$Å^3$. Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre under deposition number 917978. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
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21. Vibrational measurements were carried on a BioTools-BOMEM Chiral*IR* FT-VCD spectrophotometer equipped with dual photoelastic modulation. A sample of 7.7 mg of **1** was dissolved in 150 μL of 100% atom-D $CDCl_3$, placed in a BaF_2 cell with a pathlength of 100 μm and data were acquired at 4 cm^{-1} during 3 h. Sample stability was monitored by 300 MHz ^{1}H NMR measurements immediately prior and after vibrational measurements.
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