Research Article

Density functional theory studies of Hypaphorine from *Erythrina mildbraedii* and *Erythrina addisoniae*: structural and biological properties

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Abstract

This study was aimed at isolating hypaphorine from *Erythrina mildbraedii* Harms (Fabaceae) and *Erythrina addisoniae* Hutch. & Datzel (Fabaceae) in order to determine its structural and antibacterial effects. Density functional theory (DFT) calculations and X-ray crystallographic analysis of the isolated hypaphorine was determined. The antibacterial effects of hypaphorine against a number of Gram positive and Gram negative bacterial strains were investigated. The root mean square deviation between the experimental and calculated bond lengths and bond angles of hypaphorine were found to be 0.046 Å and 1.5° respectively. The highest occupied molecular orbital (HOMO) of hypaphorine was delocalised on the indole moiety whereas the lowest occupied molecular orbital (LUMO) was delocalised on the $-N(CH_3)_3$ group and the HOMO–LUMO gap of hypaphorine was 4.65 eV. Hypaphorine inhibited the growth of the Gram-positive bacteria tested, namely *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus* and *S. epidermidis*. The lowest minimum inhibitory concentration (MIC) value of 2 mg/mL was exhibited against *Mycobacterium smegmatis*, *Staphylococcus aureus* and *B. subtilis*. The theoretical and experimental results from this study showed that hypaphorine is capable of forming quadrupole moments thus explaining its antibacterial effects on Gram positive bacteria.

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Graphic abstract

	Hypaphorine	Tryptophan
Dipole moment (D)	8.177	3.073
Quadrupole moment (D Å)	-15.640	-5.191

Keywords Hypaphorine · *Erythrina mildbraedii* · *Erythrina addisoniae* · Crystal structure · Antibacterial · Density functional theory

1 Introduction

Hypaphorine (also known as lenticin, with the IUPAC name (2S)-3-(1*H*-indol-3-yl)-2-(trimethylazaniumyl) propanoate is an indole alkaloid (Fig. 1). It was first isolated and characterized from *Erythrina hypaphorus* Boerl. Ex Koord.-Schum in 1911 [1] and since has been found in a large number of different organisms ranging from plants to fungi, sponges and mammals. It has been isolated from plant species mainly in the Fabaceae family as well as beetroots and carrots [1–5]. Hypaphorine is structurally similar to a number of biochemically active compounds such as the plant hormone indole-3-acetic acid (IAA), tryptophan, melatonin and serotonin [5–7] (Figure 1S) and therefore, it has been found to be of potential medicinal value [4, 5]. It can act as a competitive inhibitor for a number of processes controlled by IAA [8–10].

Erythrina is a prominent source of phytoconstituents and medicinal agents [9]. They are widely known for their symbiotic relationships with mycorrhizal fungi. The presence of hypaphorine in the roots of these plants inhibit

root hair formation and stimulates curling of the roots which aids in these symbiotic relationships [7]. Hypaphorine has thus been detected in a number of ectomycorrhizal fungal species like *Pisolithus tinctorius* [1, 11, 12]. In these symbiotic relationships, hypaphorine inhibits the effect of IAA in the fungi by blocking membrane-bound IAA receptors thus inducing abnormal proliferation of hyphae that extend intracellularly into plant tissues.

Fig. 1 Hypaphorine and its atom numbering

Bacteria growing in the mycorrhizosphere of symbionts such as *P. tinctorius* may stimulate the production hypaphorine [13].

To the best of our knowledge, the crystal structure of hypaphorine and its antibacterial activity have not been investigated. Therefore, in this work, hypaphorine was isolated from *Erythrina mildbraedii* Harms (Fabaceae) and *Erythrina addisoniae* Hutch. & Datzel (Fabaceae), its crystal structure, geometric parameters and antibacterial activities were determined. The energy gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) was obtained by theoretical calculations based on DFT—optimized conformation. DFT method are used to complement experimental investigations involving natural products [12, 14, 15].

2 Experimental

2.1 Materials and bacterial strains

The following bacterial strains were obtained from Davies Diagnostics: Gram-positive bacteria: *Bacillus cereus* (ATCC 10876), *B. subtilis* (ATCC 19659), *Enterococcus faecalis* (ATCC 13047), *Mycobacterium smegmatis* mc2 155, *Staphylococcus epidermidis* (ATCC 14990) and *S. aureus* (ATCC 25923). Gram-negative bacteria: *Enterobacter cloacae* (ATCC 13047), *Escherichiacoli* (ATCC 25922), *Klebsiella oxytoca* (ATCC 8724), *K. pneumonia* (ATCC 13882), *Proteus mirabilis* (ATCC 7002), *P. vulgaris* (ATCC 6380) and *Pseudomonas aeruginosa* (ATCC 27853). All strains were confirmed purity stock cultures and maintained in the Biotechnology laboratories of the University of Johannesburg. Mueller–Hinton agar and Mueller–Hinton broth were obtained from Oxoid. Nalidixic acid was bought from Sigma.

Stem bark of *E. addisoniae* and *E. mildbraedii* were collected from trees in Yaounde (Centre region of Cameroon) and Buea (South West region of Cameroon) that had been used previously [16, 17]. The stem barks were identified and compared to existing voucher specimens of *E. addisoniae* (No. 41617/HNC) and *E. mildbraedii* (No. 50452/HNC) by Mr. Victor Nana, a botanist at the Cameroon National Herbarium (Yaoundé, Cameroon).

Column chromatography (CC) was carried out using Merck silica gel 60 PF₂₅₄ and silica gel 60, respectively. For TLC Merck precoated silica gel 60 F₂₅₄ plates were used. Spots on TLC were visualized under UV light and by spraying with anisaldehyde–H₂SO₄ reagent followed by heating.

All NMR work was done on a Bruker Avancell (400 MHz) and a Bruker Avancell (500 MHz), with TMS or residual solvent peaks as a reference.

2.2 Hypaphorine extraction and its purification

The method by Wandji and coworkers [18] was adapted. The plant material was dried, finely ground into powder and extracted at room temperature (10 g plant material/150 mL solvent) with a 50:50 methanol:chloroform solution. The solvent was replaced daily. After 3 days the pooled solvent was filtered and concentrated at 35 °C using a rotary evaporator under reduced pressure.

The crude extract was subjected to gravity column chromatography on silica gel, using a gradient solvent system of cyclohexane, CH₂Cl₂, EtOAc and MeOH in increasing proportions of the polar solvent to yield fractions. The fractions were characterized on TLC using CH₂Cl₂–MeOH, 6.5:3.5. Similar fractions were pooled and concentrated at 35 °C using a rotary evaporator. The remaining solvents were allowed to evaporate in a desiccator and the resulting crystals were subjected to 1D and 2D NMR analysis to positively identify hypaphorine [19].

2.3 Crystallography: data collection

A colourless block-like crystal with approximate dimensions 0.174 × 0.265 × 0.317 mm³ was selected under oil under ambient conditions and attached to the tip of a MiTeGen MicroMount®. The crystal was mounted in a stream of cold nitrogen at 100(1) K and centred in the X-ray beam by using a video camera. The crystal evaluation and data collection were performed on a Bruker APEXII CCD diffractometer with Mo K α radiation (λ = 0.71073 Å) and the diffractometer to crystal distance of 4.96 cm.

The initial cell constants were obtained from three series of ω scans at different starting angles. Each series consisted of 15 frames collected at intervals of 0.4° in a 6° range about ω with the exposure time of 10 s per frame. The reflections were successfully indexed by an automated indexing routine built in the APEXII program suite. The final cell constants were calculated from a set of 823 strong reflections from the actual data collection. The data were collected by using the full sphere data collection routine to survey the reciprocal space to the extent of a full sphere to a resolution of 0.75 Å. A total of 18,498 reflections were harvested by collecting 7 sets of frames with 0.5° scans in ω and ϕ with exposure times of 10 s per frame. These highly redundant datasets were corrected for Lorentz and polarization effects. The absorption correction was based on fitting a function to the empirical transmission surface as sampled by multiple equivalent measurements [20].

2.4 Computer programs used to collect data, solve and refine the structure

Data collection—BRUKER APEXII; Structure solution—SHELXS [21, 22]; Data reduction—BRUKER SAINT and SADABS [20]; Structure refinement—SHELXL [21, 22]; Structure manipulation and molecular graphics—OLEX2 [23] and Structure checking—PLATON [24].

2.5 Antibacterial tests: disk diffusion essay

The method described by Othman et al. was adapted [25]. Overnight bacterial cultures (of the 14 strains listed above) grown in Mueller–Hinton broth were diluted to match a 0.5 McFarland standard. A sterile swab was dipped into the bacterial culture and used to closely streak the bacteria onto 20 mL Mueller–Hinton agar in a petri dish. Discs containing 500 µg antibiotic or hypaphorine were placed on the seeded agar plates and the plates were incubated at 37 °C for 24 h. Each experiment was repeated five times. Zones of inhibition were measured and reported in mm.

2.6 Minimum inhibitory concentration

The minimum inhibitory concentration of hypaphorine was determined against the five bacterial strains that showed activity in the disc diffusion assay (*B. cereus*, *B. subtilis*, *M. smegmatis*, *S. aureus* and *S. epidermis*). The method reported by Andrew [26] was followed. All tests were repeated five-fold. A stock solution of hypaphorine was prepared at 32 mg/mL in methanol, and serially diluted with sterile Mueller–Hinton broth. The antibiotic nalidixic acid was also prepared (32 mg/mL) and serially diluted

in the same way to act as control. 100 µL aliquots of the serially diluted hypaphorine solutions or antibiotic dilutions were pipetted into individual wells of a microtitre plate. 100 µL of a bacterial suspension (diluted to yield an inoculum size of 106 CFU's) was added to each well. The plates were incubated at 37 °C for 16 h. 10 µL of a 0.02% (w/v) of resazurin sodium salt solution was added to each well and the plates were re-incubated at 37 °C for 2 h. Color change from blue to pink indicated live bacterial cultures. To confirm viability of the bacterial cultures 100 µL of sterile Mueller–Hinton broth was mixed with 100 µL bacterial suspension in five wells.

2.7 Computational methodology

Hypaphorine was optimised using the DFT method with the B3LYP functional [27, 28] in conjunction with the 6–311 ++G(d,p) basis set [29] for all atoms. Full optimisation, without any symmetry restrictions, was carried out in the gas phase using the tight criteria. Frequency computation of the optimised geometry was also performed to ensure that the optimised structure was a true local minimum. In order to mimic the experimental NMR conditions, hypaphorine was also optimised in water as the solvent. The solvent computations were carried out using the polarizable continuum model as developed by Tomasi's group [30, 31] in the framework of self-consistent reaction field. The optimized structure in water was used for computing chemical shifts with the Gauge-Including Atomic Orbital method [32] using shieldings of TMS computed at the same theoretical level and basis set. Gaussian 09 program [33] was used for all the computations by means of the resources provided by Gridchem [34, 35].

Table 1 ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectral data δ , multiplicity, J of hypaphorine in D_2O

Atom label- ling/ $\Delta\delta\text{c}/$	Experiment			Computed		Experiment	Computed		Chem draw	$\Delta\delta_{\text{H}}/$
			Chem draw	$\delta^{13}\text{C}$	$\Delta\delta\text{c}/$	$\delta^1\text{H}$ (m, $J=\text{Hz}$)		$\Delta\delta_{\text{H}}/$		$\Delta\delta_{\text{H}}/$
1		–		–		10.17 s	8.06 (8.47) ^a	2.11	10.88	0.68
2	1.78	124.68	122.9	131.87	9.19	7.16 s	7.27	0.11	7.18	0.08
3	3.73	107.07	110.8	117.22	10.15	–	–	–		
4	0.99	126.41	127.4	135.10	8.69	–	–	–		
5	0.52	119.42	118.9	124.52	5.10	7.61 d 8.05	8.03	0.42	7.58	0.03
6	0.16	122.04	122.2	126.16	4.10	7.17 t 8.00	7.45	0.28	6.97	0.20
7	1.86	118.14	120.0	128.45	10.31	7.24 t 8.05	7.51	0.27	7.13	0.11
8	0.91	112.01	111.1	117.81	5.80	7.48 d 8.05	7.68	0.20	7.34	0.14
9	0.28	136.12	136.4	144.42	8.30	–	–	–		
10	1.68	22.73	24.4	28.87	6.14	3.21 d 6.8	3.22, 3.40	0.01	3.29 3.04	0.05
11	1.96	78.86	16.9	87.18	8.32	3.81 t 6.8	3.81	0.00	4.82	1.01
12	0.2	171.40	171.6	177.42	6.02	–	–			
13	0.01	51.91	51.9	48.92–56.89	2.99–4.98	3.16 s	2.57–4.84	0.59–1.68	3.30	0.14

^aComputed using the M06-2X/6–311 ++G(d,p) method

Table 2 Crystallographic data of hypaphorine

Crystal data	
Chemical formula	C ₁₄ H ₁₈ N ₂ O ₂
M_r	246.30
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Temperature (K)	100
a, b, c (Å)	9.517 (4), 10.784 (4), 12.422 (4)
V (Å ³)	1274.8 (8)
Z	4
Radiation type	Mo $K\alpha$
μ (mm ⁻¹)	0.09
Crystal size (mm)	0.32 × 0.27 × 0.17
Data collection	
Diffractometer	Bruker APEX-II CCD diffractometer
Absorption correction	Numerical SADABS 2012/1 (Bruker, 2012) was used for absorption correction. $wR2(int)$ was 0.0563 before and 0.0511 after correction. The Ratio of minimum to maximum transmission is 0.9319. The $\lambda/2$ correction factor is 0.0015.
T_{min}, T_{max}	0.884, 0.949
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	18,498, 3230, 2919
R_{int}	0.042
$(\sin \theta/\gamma)_{max}$ (Å ⁻¹)	0.672
Refinement	
$R[F^2 > 2\sigma(F^2)], wR(F^2), S$	0.034, 0.079, 1.05
No. of reflections	3230
No. of parameters	171
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\Delta_{max}, \Delta_{min}$ (e Å ⁻³)	0.24, - 0.19
Absolute structure	Refined as an inversion twin.
Absolute structure parameter	0.3 (13)

3 Results and discussion

3.1 Hypaphorine from *Erythrina* genus

NMR results positively identified hypaphorine in the stem bark of both *E. addisoniae* and *E. mildbraedii*. ¹H and ¹³C NMR spectral data were in agreement with literature [18, 19] and are presented in Table 1. Computed NMR parameters are also presented in Table 1 and in general, there is a good correlation between the experimental and theoretical chemical shifts. Both species are indigenous to West Africa and are used as medicinal plants to treat a number of ailments. Isoflavonoids from this species have shown PTP1B inhibition and cytotoxic activity, which give the plant potential to be used as anticancer treatment [16, 17]. Extracts from *E. mildbraedii* are used to treat stomach problems and various diseases associated with bacterial infection, asthma, female infertility, ulcers, and inflammations [36, 37]. Plant foods from the Fabaceae family such as peanuts, chickpeas or lentils contain high levels

of hypaphorine. It was also detected in the bloodstream and in human breast milk after the consumption of food containing this compound [4].

3.2 Crystal structure and DFT results

Crystal data, data collection, structure refinement details and resolved final structure are summarized in Table 2. CCDC-1401794 contains the supplementary crystallographic data for this paper. This data can be obtained free of charge at <http://www.ccdc.cam.ac.uk>. The systematic absences in the diffraction data are consistent for the orthorhombic chiral space group $P2_12_12_1$. The e-statistics also confirm this non-centrosymmetric space group and yield chemically reasonable and computationally stable results of refinement [21, 22]. A successful solution by the direct methods in the program SHELXS [21, 22] within the crystallographic program OLEX2 [23] provide most non-hydrogen atoms from the electron density map.

Fig. 2 Packing of Hypaphorine viewed down the *b*-axis. Red dashed line indicates the hydrogen bond that directs the packing

The remaining non-hydrogen atoms are located in an alternating series of least-squares cycles in the program SHELXL [21, 22] and difference Fourier maps. All non-hydrogen atoms were refined with anisotropic displacement coefficients unless specified otherwise. C-bound H atoms are placed in geometrically idealized positions, with $C-H = 0.93-0.99 \text{ \AA}$, and are constrained to ride on their parent atoms with relative isotropic displacement coefficients, with $U_{iso}(H) = 1.2U_{eq}(C)$ for aromatic and methylene H atoms, and $U_{iso}(H) = 1.5U_{eq}(C)$ for methyl H atoms. The methyl H atoms were initially located in a different Fourier map, but since they are not involved in any hydrogen-bonding interactions they are placed in idealized positions as described above and refined as rotating groups. H atoms on N atoms are also located in a different Fourier map and since they are involved in hydrogen bonding, the positions and isotropic displacement parameters of these atoms are allowed to refine freely.

The crystal structure was also refined as a racemic twin with a Flack x parameter of 0.3(13) indicating that the ratio of the two enantiomers in the crystal structure are approximately in the ratio of 30:70. A comprehensive search of the Cambridge Structural Database [38]—CSD 2019 release with February 2019 updates) of organic and organometallic crystal structures, for a crystal structure of hypaphorine yielded one result for the zwitterion structure (IZUTUU)—our structure that we report here that had been submitted to the CSD as a private communication in 2016 [39].

Also, two related structures were published in CSD, namely L-6-Bromohypaphorine (BHYPOR) [40] and L-hypaphorine hydroiodide (PAMRUQ) [41].

The packing diagram of hypaphorine is depicted in (Fig. 2). The hypaphorine molecules pack end-to-end in layers diagonal to the packing axis (when viewed down the *b*-axis). When viewed exactly down the *b*-axis, molecules pack in an alternate stacking manner. This packing arrangement results from the hydrogen bonding that links these alternate layers through one strong hydrogen bond between N_2 of the one molecule and O_2 of the alternately stacked molecule. Figures 2 and 3 shows the hydrogen bonding interaction between two hypaphorine molecules—indicated by a dashed red line.

The optimized geometry of hypaphorine is illustrated in Figure 2S (supplementary material). The comparison of the calculated bond angles and bond lengths and the corresponding solid state values are listed in Table 1S. The DFT data were compared with the X-ray data (Table 2, Fig. 4) and it was found that the root mean square deviation (RMSD) between the experimental and calculated bond lengths and bond angles of hypaphorine are found to be $0.046 \text{ \AA}$ and 1.5° respectively. The difference in values may be due to the fact that the experimental results were obtained at solid phase and the molecular rotations were restricted, while the theoretical results were obtained for a single molecule in the gas phase without any intermolecular interactions.

Fig. 3 Molecular structure diagram of hypaphorine showing the strong hydrogen bond (indicated by dashed red line) that directs the packing

Fig. 4 Crystal structure of hypaphorine

The frontier molecular orbitals and their characteristics are used to explain reactions in a conjugated system and to predict the reactive position in π -electron systems [42]. The highest occupied molecular orbital (HOMO) represents the ability to donate an electron and it is correlated to the ionization potential. The lowest occupied molecular orbital (LUMO) represents the ability to accept an electron which is correlated to electron affinity. The energy gap between the HOMO and LUMO levels is associated to molecular chemical stability [43, 44]. The HOMO and LUMO

surfaces of hypaphorine are illustrated in Fig. 5. The HOMO is delocalised in the indole moiety whereas the LUMO is delocalised on the $-\text{N}(\text{CH}_3)_3$ group. The HOMO–LUMO gap of hypaphorine is 4.65 eV and the reactivity descriptors are also included Table 3. The natural charges on the atoms are provided in Table 2S.

The simulated IR spectrum of hypaphorine in the gas phase is presented in Fig. 6. Two stretching vibrations namely the uncorrected N–H stretching and C=O stretching are found at 3673 cm^{-1} and 1727 cm^{-1} , respectively.

Fig. 5 Frontier molecular orbitals of hypaphorine (HOMO and LUMO) computed in the gas phase

Table 3 Energy of HOMO and LUMO levels and derived reactivity descriptors of hypaphorine

Parameter	Equations	Value
E_{HOMO} (eV)		- 5.61
E_{LUMO} (eV)		- 0.96
HOMO–LUMO gap (eV)	$\Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$	4.65
Ionisation potential (I) (eV)	$I = -E_{\text{HOMO}}$	5.61
Electron affinity (A) (eV)	$A = -E_{\text{LUMO}}$	0.96
Electronegativity (χ) (eV)	$\chi = -\mu$	3.29
Chemical potential (μ) (eV)	$\mu = -(I + A)/2$	- 3.29
Chemical hardness (η) (eV)	$\eta = I - A$	2.32
Chemical softness (S) (eV^{-1})	$S = 1/\eta$	0.22
Electrophilicity index (ω) (eV)	$\omega = \mu^2/2\eta$	2.32

3.3 Antibacterial results

The antibacterial assay results are presented in Table 4. Pure hypaphorine inhibited the growth of all the Gram-positive bacteria tested, namely *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Mycobacterium smegmatis*, except *Enterococcus faecalis*. It was found that hypaphorine did not affect the growth of any of the Gram-negative bacteria tested. Njamen and colleagues [36] found that pterocarpanes from *E. mildbraedii* showed antimicrobial effects against *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Streptococcus mutans*, and the Gram negative *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*, indicating the

Fig. 6 Simulated IR spectrum of hypaphorine in the gas phase

Table 4 Antibacterial characteristics of hypaphorine

Bacterial Strain	Zone of Inhibition (mm)	MIC (mg/mL) of Hypaphorine	MIC (mg/mL) of nalidixic acid
<i>B. cereus</i>	10.9 ± 1.2	8	2
<i>B. subtilis</i>	21.9 ± 0.7	2	Below 1
<i>M. smegmatis</i>	15.8 ± 0.7	4	Above 16
<i>S. aureus</i>	12.1 ± 0.9	2	2
<i>S. epidermidis</i>	13.2 ± 1.5	4	2

possibility for synergistic effects from a number of compounds found in the plant.

Tryptophan and arginine-rich peptides are known to have antimicrobial properties [44]. It has been proposed that tryptophan (structurally similar to hypaphorine) has a complicated interaction with the bilayer structure of membrane structures since it has a significant quadrupole moment that makes it hydrophilic, even though its structure suggests it should be hydrophobic. Tryptophan tends to locate itself in the interface region between the hydrophilic heads and hydrophobic tails in a membrane. Peptides rich in tryptophan and arginine can affect bacterial cells by either destabilizing the cell membrane or by binding to bacterial DNA and so block macromolecule replication [45].

Duponnois and Plenchette [13] proposed that bacteria in and around a mycorrhizal symbiotic relationship may stimulate hypaphorine production, but it may also be possible that the hypaphorine produced by either the plant or fungi may alter the bacterial populations in the mycorrhizosphere to suit them more. As such, the results in Table 4 are not too surprising considering that Gram-negative *Pseudomonas* bacteria are the dominant genus in the mycorrhizosphere of *Pinus sylvestris*–*Suillus bovinus* [46]. Other researchers [46, 47] found that general bacteria numbers are higher inside the mycorrhizosphere of various species of plants and fungi, probably due to the high concentration of organic matter to obtain nutrients from. Frey-Klett and colleagues [48] reported that *Pseudomonas* bacteria can mobilize iron or solubilize phosphorus from inorganic sources. In contrast, Barbieri and colleagues [49] found that *Staphylococcus pasteurii* bacteria released strong antifungal volatile organic compounds against *Tuber borchii* fungi. It would, therefore, appear that hypaphorine may play a role in maintaining the balance of specific Gram-negative and positive bacteria populations to suit the needs of the fungal symbionts in mycorrhizal relationships.

Of interest from this study is the fact that hypaphorine showed inhibitory properties against *Mycobacterium smegmatis* and *Staphylococcus aureus*. From Table 4, it

Table 5 Dipole moment, quadrupole moment, logP and logS of hypaphorine and tryptophan

	Hypaphorine	Tryptophan	nalidixic acid
Dipole moment (D)	8.177	3.073	
Quadrupole moment (D Å)	– 15.640	– 5.191	
logP ^a	– 0.54	– 1.10	0.95
logS ^a	– 5.07	– 2.18	– 2.00

^alogP and logS were estimated using ALOGPS 2.1 [55]

seems that mainly Gram-positive bacteria are susceptible to the compound. *Mycobacterium tuberculosis* is the causative agent of TB and since 2013, the WHO has estimated that 9 million people fell ill with TB and that over a million died from the disease [50]. TB is the second largest killer after HIV; moreover, TB/HIV co-infections have been a regular feature in countries plagued by the HIV. Methicillin-resistant *Staphylococcus aureus* (MRSA), is also responsible for life-threatening infections usually acquired in major hospitals and other health care facilities [51].

Whilst several studies have previously looked at antibacterial activities of crude plant extracts from *Erythrina* species [36, 51, 52] showed that isoflavonoids from *Erythrina variegata* are potent against MRSA with MIC values below 100 µg/mL. From Table 4, it can be noted that hypaphorine has a much lower potency at 2 000 µg/mL against *S. aureus* which is not comparable to the above mentioned isoflavonoids. Arpha and colleagues [53] came to a similar conclusion when testing hypaphorine against *M. tuberculosis*, with an MIC above 50 mg/mL. This leaves scope for investigating the synergistic effects of compounds such as hypaphorine and isoflavonoids against problematic bacterial strains. Possible additional compounds with lesser side effects could be useful for future therapeutic applications. It is known that hypaphorine is related to the naturally occurring indole-3-acetic acid; possible synthetic structural analogs can be generated and evaluated to further heighten bioactive properties against MRSA and *M. tuberculosis*.

Dipole moment, quadrupole moment, hydrophobicity (logP) and solubility in water (logS) have been used as molecular parameters to relate to biological properties of compounds [54, 55]. The estimated values of these parameters for hypaphorine and tryptophan are collected in Table 5. It can be deduced that hypaphorine has a larger dipole moment and quadrupole moment than tryptophan. Hypaphorine is also expected to be less hydrophobic and has lower solubility in water than tryptophan.

4 Conclusion

Hypaphorine was isolated from *E. addisoniae* and *E. mildbraedii*. The structure of hypaphorine shows that the non-polar double ring structure tends to lie on one plane and a polar carboxyl group stands out from that plane, allowing for a quadrupole moment. In theory this should allow hypaphorine to have similar antibacterial effects as tryptophan that is, it should be capable of inserting itself into the bilayer structure of bacterial cell membranes where it can disrupt the membranes.

The ecological impact of hypaphorine in a symbiotic context with bacteria and fungi will have to be investigated. A clear determination will have to be made of where it is originally produced—in the fungal hyphae, plant roots or simultaneously in both. Tests will have to be done on how the presence of hypaphorine influences subsequent production levels in both symbionts, if and how hypaphorine is transported in both symbionts, and how the presence of hypaphorine affects the population numbers of mixed soil bacteria.

For the medicinal properties of hypaphorine, future studies will be to investigate the actual mechanisms by which it affects both *M. smegmatis* and *S. aureus* and also studying the compound's effects on the actual *M. tuberculosis* and MRSA. By identifying small molecules from the natural (or synthetic) products arsenal against pathogenic bacteria, a window of opportunity is opened to gain insights into bioactive compounds destined against problematic microbes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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