Phytochemistry Letters 10 (2014) 28–31

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

3-Oxo-14α,15α-epoxyschizozygine: A new schizozygane indoline alkaloid from *Schizozygia coffaeoides*

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ARTICLE INFO

Article history:
Received 27 May 2014
Received in revised form 7 July 2014
Accepted 10 July 2014
Available online 23 July 2014

Keywords:
Schizozygia coffaeoides
Schizozygane indoline alkaloid
3-Oxo-14α,15α-epoxyschizozygine
Dimeric anthraquinone
Cassiamin A
Cassiamin B

ABSTRACT

The stem bark extract of *Schizozygia coffaeoides* (Apocynaceae) showed moderate antiplasmodial activity (IC_{50} = 8–12 μg/mL) against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. Chromatographic separation of the extract led to the isolation of a new schizozygane indoline alkaloid, named 3-oxo-14α,15α-epoxyschizozygine. In addition, two dimeric anthraquinones, cassiamin A and cassiamin B, were identified for the first time in the family Apocynaceae. The structures of the isolated compounds were deduced on the basis of spectroscopic evidence. The schizozygane indole alkaloids showed good to moderate antiplasmodial activities (IC_{50} = 13–52 μM).

1. Introduction

Schizozygia coffaeoides (Boj.) Baill. (Family, Apocynaceae) is a monotypic shrub or small tree indigenous to Central and East Africa (Barink, 1983). Traditionally, the plant has been used for the treatment of skin diseases in Kenya (Kokwaro, 1993). Biological evaluation of the crude extract of the leaves showed high antifungal activity (Kariba et al., 2001). Previous phytochemical investigations of the leaves have resulted in the isolation of the *N*-acyl indoline alkaloid schizozygine (**1**, Fig. 1) along with schizogamine, schizogaline, isoschizogamine and isoschizogaline (Renner and Kernweisz, 1963). The structure of isoschizogamine was subsequently revised from a 6-membered to 5-membered lactam ring (Hajicek et al., 1998). Similarly Kariba et al. (2002) revised the structure of isoschizogaline, and also identified a new schizozygane indoline alkaloid 6,7-dehydro-19β-hydroxyschizozygine as the antifungal principle of the leaves.

We have investigated the stem and roots of *S. coffaeoides* and report here the isolation of a new schizozygane indoline alkaloid, named 3-oxo-14α,15α-epoxyschizozygine (**2**, Fig. 1) along with known compounds, including the dimeric anthraquinones, cassiamin A and cassiamin B (Koyama et al., 2001). The antiplasmodial activities of the crude stem bark extract and four of the schizozygane indoline alkaloids are also reported.

2. Results and discussion

The major compound of the stem bark of *S. coffaeoides* was identified as schizozygine (**1**) which has previously been isolated from the roots and leaves of this plant (Renner and Kernweisz, 1963; Kariba et al., 2002). The HRMS of compound **2** showed a molecular ion peak at *m/z* 366.1209 corresponding to the molecular formula $C_{20}H_{18}O_5N_2$. Comparison of the NMR spectra of **2** with that of **1** (Table 1) showed that **2** has the basic skeleton of schizozygine (**1**). Thus the ^{1}H NMR spectrum of **2** showed the presence of two *para*-oriented aromatic protons resonating at δ_H 6.68 (*s*) and 7.68 (*s*) corresponding to H-9 and H-12, respectively. The HMBC correlation of H-7 with C-2, C-8, C-9 and C-13, and that of H-9 with C-7, C-10, C-11, and C-13 (Table 1) established the presence of an indole moiety in compound **2**. The substituent on the aromatic ring was shown to be a methylenedioxy group (δ_C 102.0) whose protons appeared as two mutually coupled

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http://dx.doi.org/10.1016/j.phytol.2014.07.003
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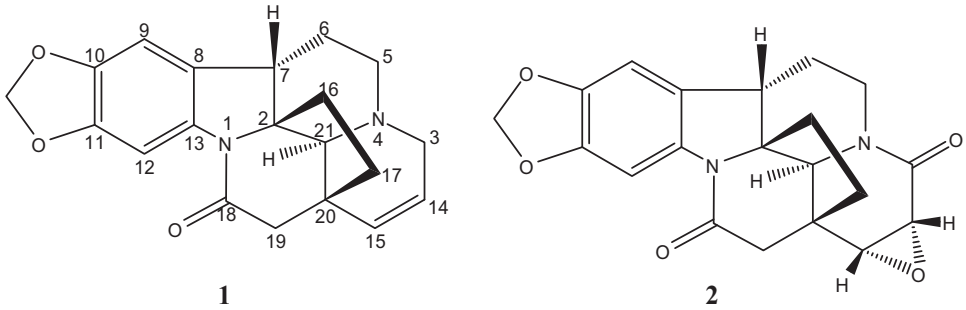

Fig. 1. Structures of compounds **1** and **2**.

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data together with HMBC correlation for **1** and **2** in CD_2Cl_2.

C	1			2		
	δ_H (*m*, *J* in Hz)	δ_C	HMBC	δ_H (*m*, *J* in Hz)	δ_C	HMBC
2		72.6			73.0	
3a	2.80 (*d*, 16.8)	53.5	C-15, C-14		166.2	
3b	3.36 (*d*, 16.9)					
5a	2.27 (*m*)	50.1		3.87 (*m*)	41.4	C-6, C-7
5b	3.03 (*bs*)			3.21 (*td*, 13.8, 3.0)		C-7
6a	2.04 (*m*)	25.8	C-2, C-5, C-7	1.77 (*qd*, 13.6, 3.9)	27.0	C-2
6b				2.23 (*m*)		
7	3.20 (*t*, 6.7)	42.1	C-2, C-5, C-6, C-8, C-9, C-13, C-16, C-21	3.16 (*dd*, 12.6, 5.5)	44.2	C-2, C-5, C-6, C-8, C-9(w), C-13, C-16
8		125.4			124.8	
9	6.66 (*d*,1.1)	104.2	C-13, C-12, C-11, C-10, C-7	6.68 (*s*)	104.4	C-8, C-10, C-11, C-13
10		144.2			145	
11		147.1			147.7	
12	7.61 (*s*)	98.4	C-8, C-9, C-10, C-11, C-13	7.68 (*s*)	99.1	C-10, C-11, C-13
13		137.0			135.7	
14	5.57 (*ddd*, 10.0, 4.4, 2.0)	123.8	C-3, C-20	3.53 (*d*, 4.0)	52.2	C-3, C-15
15	5.73 (*dt*, 9.9, 2.2)	130.2	C-14	3.48 (*d*, 4.0)	58.0	C-20, C-21, C-14
16a	2.29 (*m*)	38.6	C-2, C-17, C-20	2.50 (*m*)	40.8	C-2, C-21
16b				2.00 (*br s*)		
17a	1.86 (*ddd*, 12.8, 8.6, 5.3)	37.6	C-2, C-19, C-21	2.01 (*m*)	30.9	C-7
17b	2.05 (*m*)		C-14, C-21	1.92 (*m*)		
18		168.9			165.3	
19a	2.45 (*dd*, 18.0, 2.8)	46.9	C-15, C-17, C-18, C-20	2.92 (*dd*, 17.6, 2.4)	45.2	C-15, C-17, C-18, C-20
19b	2.61 (*d*, 18.0)		C-17, C-18, C-20, C-21	2.77 (*d*, 17.6)		
20		44.7			46.3	
21	2.25 (*s*)	68.0	C-2, C-17, C-20	3.51 (*br s*)	58.8	C-7, C-16, C-17
OCH_2O	5.917 (*d*,1.4)	101.7	C-10, C-11	5.948 (*d*, 1.3)	102.0	C-10, C-11
	5.923 (*d*,1.4)			5.952 (*d*, 1.3)		

doublets at δ_H 5.948 and 5.952 (J = 1.3 Hz). In support of this, the methylenedioxy protons showed HMBC correlation with C-10 and C-11. Furthermore, H-7 (δ_H 3.16) correlates with C-2 (δ_C 73.0), C-5 (δ 41.4) and C-6 (δ_C 27.0) while one of the C-5 protons correlates with C-6 and C-7, which is in agreement with a piperidine ring being fused to the indole moiety at the C-2/C-7 junction as in schizozygine (**1**).

Further annulation at N-1/C-2 and C-21/N-4, as well as the presence of an ethylene bridge between C-2 and C-20 was evident from the NMR spectra (Table 1). Thus, in the HMBC spectrum, H-21 (δ_H 3.51) correlates with C-7 (δ_C 44.2), C-16 (δ_C 40.8) and C-17 (δ_C 30.9). Also H-19a (δ 2.45) correlates with C-15 (δ 58.0), C-17 (δ 30.9), the amidic carbonyl C-18 (δ_C 165.3) and C-20 (δ_C 46.3). That H-7, CH_2-16 and CH_2-17 are on the same face (β-orientation) as in **1** was clearly evident from NOE interactions among these protons in the NOESY spectrum (Fig. 2). The chemical shift values of the amidic carbonyl C-18 (δ_C 165.3), C-2 (δ_C 73.0) and C-21 (δ_C 46.3) are consistent with a six-membered lactam ring with an ethylene bridge between C-2 and C-20 as in schizozygine (Fig. 1).

The only difference between **2** and schizozygine (**1**) is the presence of an additional amidic carbonyl (δ_C 166.2) at C-3 [supported by *ca.* 10 ppm low frequency shift of C-5 and C-21 in the ^{13}C NMR spectrum as a result of change of the amine in **1** into amide in **2** (Pretsch et al., 2010; Kalinowski et al., 1984)] and the replacement of the double bond between C-14 and C-15 with an epoxy group. This was evident from the ^{1}H NMR spectrum which showed the presence of two mutually coupled doublets (J = 4.0 Hz) resonating at δ_H 3.53 (H-14) and δ_H 3.48 (H-15), with the corresponding carbon atoms appearing at δ_C 52.2 and 58.0, respectively. In agreement with this substructure, the HMBC spectrum showed that H-15 correlates with C-20 (δ 46.3) and C-21 (δ 58.8), while H-14 correlates with C-3 (δ 166.2). Therefore this compound was characterized as 3-oxo-14α,15α-epoxyschizozygine (**2**) which is a new compound. The CD spectrum of **2** showed first positive (at λ 320 nm) and then negative (at λ 272 nm) Cotton effects, as in **1** and the other related schizozygane alkaloids of this plant (Stephens et al., 2007), which is consistent with 2*R*,7*R*,20*S*,21*S* configuration. In the NOESY spectrum, H-15 showed NOE interaction with H-14, CH_2-17 and H-19β (Fig. 2), indicating that they are on the same face, β-oriented (14*R*, 15*R* configuration); the epoxy group is then α-oriented. This compound is a new addition to the schizozygane indole alkaloids, a small group of *N*-acyl indoline alkaloids having the uncommon ethylene bridge at C2-C20, which are more or less restricted to *S. coffaeoides*.

The other compounds isolated from the stem bark were identified as the known alkaloids schizozygine (**1**), schizogaline,

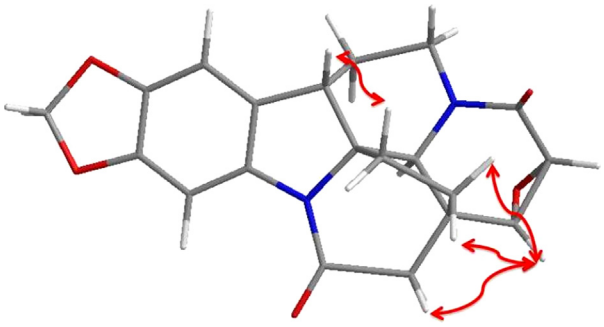

Fig. 2. Some important NOE correlations in compound **2**.

isoschizogaline and 6,7-dehydro-19β-hydroxyschizozygine. The NMR data generated here supports the revision of the structure of isoschizogaline from having a six-membered lactam ring to a five-membered ring as proposed by Kariba et al. (2002). Similar phytochemical investigation of the roots of this plant resulted in the identification of the new compound 3-oxo-14α,15α-epoxyschizozygine (**2**), along with the known alkaloids schizozygine (**1**), isoschizogamine and 6,7-dehydro-19β-hydroxyschizozygine. In addition, the dimeric anthraquinones cassiamin A and cassiamin B (Koyama et al., 2001) were identified from the roots of *S. coffaeoides*. This is the first report of the occurrence of the dimeric anthraquinones in the family Apocynaceae.

As the result of restricted rotation at the biaryl linkage, the CD spectrum of cassiamin A showed a positive Cotton effect (CE) at 295 nm and a negative one at 275 nm; while cassiamin B showed the opposite, a negative first at 305 nm and then a positive at 289 nm CE. That the Cotton effects are opposite in these compounds suggests that the configuration in these two dimers is opposite. However, to determine the absolute configurations in these compounds, advanced quantum chemical CD calculations as described by Bringmann et al. (2007) may be required.

The distribution of compounds in different parts of *S. coffaeoides* is summarized in Table 2. These include six alkaloids and two dimeric anthraquinones. Among these, the new compound, 3-oxo-14α,15α-epoxyschizozygine (**2**), occurs in both the stem and roots. Schizozygine (**1**), 6,7-dehydro-19β-hydroxyschizozygine, schizogaline and isoschizogaline were previously reported from the leaves and roots (Kariba et al., 2002; Renner and Kernweisz, 1963). In this study their occurrence in the stem has been established. Isoschizogamine was reported by Hajicek et al. (1998) from the twigs, while its occurrence in the roots has been established in this study. The anthraquinone dimers cassiamin A and cassiamin B were isolated from the roots but were not detected in the other parts of the plant (Table 2).

The stem bark extract of *S. coffaeoides* showed antiplasmodial activity against the D6 (IC_{50} = 11.2 ± 1.6 μg/mL) and W2 (IC_{50} = 8.4 ± 1.5 μg/mL) strains of *Plasmodium falciparum* while the four schizozygane alkaloids isolated from this plant had IC_{50} = 13–52 μM, (Table 2). In describing *in vitro* antiplasmodial activities of natural products, a pure compound is considered inactive when it shows $IC_{50} > 200$ μM, whereas those with an IC_{50} of 100–200 μM have low activity; IC_{50} of 20–100 μM, moderate activity; IC_{50} of 1–20 μM good activity; and $IC_{50} < 1$ μM excellent/potent antiplasmodial activity (Batista et al., 2009). Similarly, activities of crude extracts are categorized as $IC_{50} < 10$ μg/mL, good activity; IC_{50} of 10–50 μg/mL, moderate activity; IC_{50} of 50–100 μg/mL, low activity; and $IC_{50} > 100$ μg/mL, inactive (Basco et al., 1994). Based on this classification, the stem bark extract showed moderate activity against the D6 and good activity against W2 strains of *P. falciparum*. The four schizozygane alkaloids showed good to moderate activities.

3. Experimental

3.1. General

The ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra were acquired with a Bruker AVANCE III NMR spectrometer using TMS as the internal standard. COSY, HMQC and HMBC spectra were obtained using the standard Bruker software (TopSpin). EI-MS: direct inlet, 70 eV on micromass GC-TOFmicro mass spectrometer (micro mass Wythenshawe, Waters Inc., UK). HR-MS: direct inlet, on Q-TOF (time of flight mass spectrometer equipped with an electronspray ionization (ESI)). UV/VIS ($CHCl_3$) spectra were recorded using a SPECORD S600 Spectrophotometer. The CD ($CHCl_3$) spectra were obtained using a JASCO J-715 spectropolarimeter.

3.2. Plant material

The stem bark and the roots of *S. coffaeoides* were collected in January 2009 from Shimba Hills, Kenya. The plant specimen was identified by Mr. Simon Mathenge of the School of Biological Sciences, University of Nairobi, where a voucher specimen (AYT-SM-036-2009) was deposited.

3.3. Extraction and isolation from the stem bark of S. coffaeoides

The air dried and ground stem bark (6 kg) of *S. coffaeoides* was extracted with dichloromethane/methanol (1:1) by cold

Table 2
Occurrence in various parts and the antiplasmodial activities of schizozygane indoline alkaloids of *Schizozygia coffaeoides* against the D6 and W2 strains of *Plasmodium falciparum*.

Test sample	IC_{50} in μM		
	Plant part	D6	W2
Crude extract[a]	Stem	11.2 ± 1.6	8.4 ± 1.5
Schizozygine (**1**) (Kariba et al., 2002; Renner and Kernweisz, 1963)	Stem, roots, leaves	33.6 ± 2.5	26.8 ± 1.9
3-Oxo-14α,15α-epoxyschizozygine (**2**)	Roots, leaves	38.3 ± 3.0	51.6 ± 5.4
6,7-Dehydro-19β-hydroxyschizozygine (Kariba et al., 2002)	Roots, leaves	19.1 ± 1.6	29.1 ± 2.7
Schizogaline (Renner and Kernweisz, 1963)	Roots, leaves	23.2 ± 1.2	13.1 ± 0.9
Schizogamine (Renner and Kernweisz, 1963)	Leaves	NT	NT
Isoschizogamine (Renner and Kernweisz, 1963; Hajicek et al., 1998)	Roots, leaves, twigs	NT	NT
Isoschizogaline (Renner and Kernweisz, 1963; Kariba et al., 2002)	Roots, leaves, stem	NT	NT
Cassiamin A	Roots	NT	NT
Cassiamin B	Roots	NT	NT
Chloroquine		0.013 ± 0.001	0.237 ± 0.031
Mefloquine		0.00478 ± 0.00039	0.01966 ± 0.0011

NT, not tested.
[a] IC_{50} values for crude extracts are given in μg/mL; IC_{50} values given as a mean from 5 measurements.

exhaustive percolation to give a dark brown crude extract (52 g) after concentration. A portion (50 g) of the extract was subjected to column chromatography on silica gel (600 g) with gradient elution using *n*-hexane containing increasing amounts of ethyl acetate. A total of 256 eluents each of *ca.* 250 ml were collected.

Purification of fraction 25 (7% ethyl acetate in *n*-hexane) using PTLC (dichloromethane/ethyl acetate: 1:1) yielded a purple amorphous solid of 6,7-dehydro-19β-hydroxyschizozygine (25 mg). Fraction 30 (7–9% ethyl acetate in *n*-hexane) was recrystallized from n-hexane/dichloromethane to give white crystals of schizozygine (**1**, 5.0 g). The combined fractions 16–29 (7–9% ethyl acetate in *n*-hexane) were subjected to PTLC (dichloromethane/ethyl acetate: 1:1) to afford a white amorphous solid of schizogaline (25 mg). Fractions 31–44 (10–25% ethyl acetate in *n*-hexane) were combined and subjected to further column chromatography on silica gel (80 g), eluting with *n*-hexane containing increasing percentages of ethyl acetate. The 15 and 40% ethyl acetate in *n*-hexane eluents were further purified by PTLC (dichloromethane/ethyl acetate/methanol: 4.5:4.5:1) to yield isoschizogaline (20 mg) and 3-oxo-14α,15α-epoxyschizozygine (**2**, 15 mg), respectively.

3.4. Extraction and isolation from the roots of S. coffaeoides

The air dried and ground roots (6 kg) of *S. coffaeoides* were extracted using ethyl acetate by cold exhaustive percolation to give a brown crude extract (62 g) after concentration. A portion (50 g) of the extract was subjected to column chromatography on silica gel (600 g), eluting with *n*-hexane containing increasing percentage of ethyl acetate. A total of 260 fractions (each *ca.* 250 ml) were collected and combined into 26 fractions based on their TLC profile.

Purification of fraction 14 (5% of ethyl acetate) using PTLC (dichloromethane/ethylacetate: 1:1) yielded 6,7-dehydro-19β-hydroxyschizozygine (15 mg). Fraction 18 (6–15% ethyl acetate) was recrystallized from *n*-hexane/dichloromethane to give white crystals of schizozygine (**1**, 5 g). Fraction 22 (25% of ethyl acetate) was subjected to MPLC on silica gel (45 g) eluting with *n*-hexane containing increasing percentage of ethyl acetate. Fraction 6 (15% ethyl acetate) yielded a yellow amorphous solid of cassiamin B (25 mg). Further purification of fraction 23 (30–35% of ethyl acetate in *n*-hexane) on Sephadex LH-20 (methanol/dichloromethane: 1:1) gave a yellow amorphous solid of cassiamin A (50 mg).

Fraction 24 (50% ethyl acetate in *n*-hexane) was purified by PTLC (dichloromethane/ethyl acetate/methanol: 4.5:4.5:1) to afford a white amorphous solid 3-oxo-14α,15α-epoxyschizozygine (**2**, 25 mg). Fraction 25 (50% ethyl acetate in *n*-hexane) was applied on Sephadex LH-20 and further purified by PTLC (dichloromethane/ethyl acetate/methanol: 4.5:4.5:1) to afford a white amorphous solid of isoschizogamine (25 mg).

3.4.1. Schizozygine (*1*)

White amorphous solid. UV ($CHCl_3$) λ_{max} nm (log ϵ) 270 (4.1), 316 (3.6). $[\alpha]_D$ +7.6° (*c* 0.001, CH_2Cl_2). CD (CH_2Cl_2) λ nm ($\Delta\epsilon$; M^{-1} cm^{-1}): $(+6.52)_{318}$; $(-7.34)_{276}$. ^1H and ^{13}C NMR (Table 1). EIMS *m/z* (rel. int.): 336 $[M]^+$ (100). HRMS *m/z* $[M]^+$ 336.1474 $C_{20}H_{20}O_3N_2$ (calculated mass 336.1474).

3.4.2. 3-Oxo-14α,15α-epoxyschizozygine (*2*)

White amorphous solid, UV ($CHCl_3$) λ_{max} nm (log ϵ): 264 (3.7), 311 (2.6). CD (CH_2Cl_2, λ nm ($\Delta\epsilon$; M^{-1} cm^{-1}): $(+6.3)_{320}$; $(-10.7)_{272}$. ^1H and ^{13}C NMR (Table 1). EIMS *m/z* (rel. int.): 366 ($[M]^+$,100), 367 (25). HRMS $[M]^+$ found *m/z* 366.1209 $C_{20}H_{18}O_5N_2$ (calculated mass 366.1216).

3.4.3. Cassiamin A

Yellow amorphous solid, UV ($CHCl_3$) λ_{max} nm (log ϵ): 262 (4.0), 447 (2.3). CD λ nm ($\Delta\epsilon$; M^{-1} cm^{-1}): $(+2.2)_{295}$; $(-4.6)_{275}$. ^1H and ^{13}C NMR (Cheng et al., 2004). HRMS $[M]^+$ *m/z* 522.0954 $C_{30}H_{18}O_9$ (calculated mass 522.0950).

3.4.4. Cassiamin B

Yellow amorphous solid, UV ($CHCl_3$) λ_{max} nm (log ϵ): 293 (4.2), 455 (2.6). CD λ nm ($\Delta\epsilon$; M^{-1} cm^{-1}): $(-2.5)_{304}$; $(+1.4)_{289}$. ^1H and ^{13}C NMR (Cheng et al., 2004). EIMS *m/z* (rel. int.): 538 $[M]^+$ (100), 539 (40). HRMS $M]^+$ *m/z* 538.0917 $C_{30}H_{18}O_{10}$ (calculated mass 538.0900).

3.5. In vitro antiplasmodial activity

The crude extracts and pure compounds were assayed using a non-radioactive assay technique as described by Smilkstein et al. (2004) with modifications (Juma et al., 2011; Yenesew et al., 2012).

Acknowledgments

Yoseph Atilaw is grateful to the German Academic Exchange Services (DAAD) for a scholarship which was offered through the Natural Products Research Network for Eastern and Central Africa (NAPRECA). We are also grateful to the International Science Program (ISP, Sweden) for research grant (KEN-02).

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