Optical Resolution and Structure Determination of New Indolizidine Alkaloids from *Elaeocarpus sphaericus*

by **Chang-Xin Zhou**[a]), **Xue-Yao Wang**[a]), **Jian-Xia Mo**[a]), **Jian Zhang***[b]), and **Li-She Gan***[a])

[a]) Institute of Modern Chinese Medicine (IMCM), College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, P. R. China (phone: +86-571-88208456; fax: +86-571-88208457; e-mail: lsgan@zju.edu.cn)

[b]) Institute of Medical Science, School of Medicine, Shanghai Jiaotong University, Shanghai 200025, P. R. China (e-mail: jian.zhang@sjtu.edu.cn)

Two new indolizidine alkaloids, (±)-3-oxoisoelaeocarpine (**1**) and (±)-elaeocarpine *N*-oxide (**2**), along with three known alkaloids, (±)-isoelaeocarpine (**3**), (±)-elaeocarpine (**4**), and (−)-isoelaeocarpiline (**5**), were isolated from an EtOH extract of the branches and leaves of *Elaeocarpus sphaericus*. The structures of these compounds were determined by spectroscopic and chemical methods. Furthermore, enantiomers of compounds **1** and **3** were separated on a chiral *CD-Ph* column, and their absolute configurations were determined by TD-DFT (=time-dependent density-functional theory) quantum-chemical calculations of their electronic circular dichroism (ECD) spectra.

Introduction. – The genus *Elaeocarpus* (Elaeocarpaceae) comprises *ca.* 200 species distributed over tropical and subtropical regions of East Asia, southwest Pacific, and Oceania [1]. The earliest phytochemical investigations around 1970 have demonstrated that indolizidine alkaloids are characteristic components of this genus [2]. Up to date, besides minor triterpenoids [3], flavonoids [4], and phenolic glycosides [5], 28 structurally diversified alkaloids were reported from eight species of this genus [1][6]. Apart from one indole alkaloid [2f] and two pyrrolidine alkaloids [6d], most of these alkaloids are indolizidine alkaloids which have demonstrated promising affinity for the human δ-opioid receptor [6b–6e].

E. sphaericus is a tall tree growing in tropical areas of south China. Previous pharmacological studies on the extracts have showed antimicrobial activity [7] and efficacy against bronchial asthma [8]. Seven indolizidine alkaloids out of the 28 alkaloids mentioned above have been isolated from *E.* sphaericus [2g]. In the current study, two new indolizidine alkaloids, (±)-3-oxoisoelaeocarpine (**1**) and (±)-elaeocarpine *N*-oxide (**2**), together with three known ones, (±)-isoelaeocarpine (**3**), (±)-elaeocarpine (**4**), and (−)-isoelaeocarpiline (**5**), were isolated from an EtOH extract of branches and leaves of *E. sphaericus* (see *Fig. 1*). The structures of the new compounds were identified by a combination of IR, UV, MS, and NMR techniques, as well as by a chemical correlation. Furthermore, enantiomeric mixtures of **1** and **3** were successfully separated with a chiral *CD-Ph* column, and the respective absolute configurations were determined by TD-DFT quantum-chemical calculations of their electronic circular dichroism (ECD) spectra.

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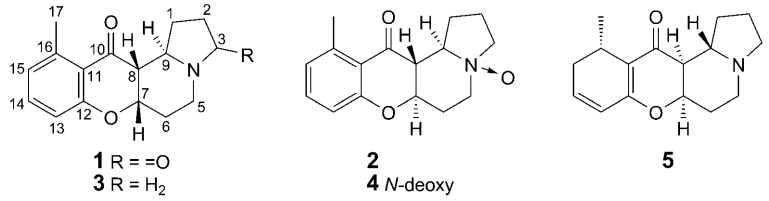


Fig. 1. *Structures of compounds* **1**–**5** *from* E. sphaericus

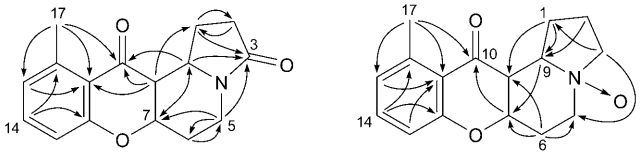
Results and Discussion. – Compound **1** was obtained as a white amorphous powder, and the molecular formula was determined as $C_{16}H_{17}NO_3$ according to its HR-ESI-MS *pseudo*-molecular-ion peak at *m*/*z* 272.1286 ($[M+H]^+$; calc. 272.1281). The IR absorption band at 1683 cm^{-1} and the UV absorption bands at 326 and 258 nm indicated the presence of an aryl ketone chromophore, similar to the phenyl indolizidine *Elaeocarpus* alkaloids [2d]. The ^{1}H-NMR spectrum of compound **1** (*Table*), similar to that of (±)-isoelaeocarpine (**3**) and (±)-elaeocarpine (**4**), showed a Me *singlet* at δ(H) 2.60, three aromatic H-atom signals between δ(H) 6.87 and 7.40, and a narrow *doublet* at δ(H) 4.77 assignable to H–C(7). The ^{13}C-NMR and HSQC spectra suggested the presence of a benzene ring (δ(C) 164.2, 143.8, 136.6, 126.3, 119.4, 117.3), two C=O groups (δ(C) 195.1, 176.5), three CH groups (δ(C) 75.3, 55.4, 54.7), four CH_2 groups (δ(C) 23.4, 29.3, 30.9, 35.8), and one Me group (δ(C) 23.3). Comparison of the ^{1}H- and ^{13}C-NMR data with those of (±)-isoelaeocarpine (**3**) revealed that compound **1** has an additional CO group at the expense of a CH_2 group unit in **3**.

Table. *NMR Data of Compounds* **1** *and* **2** *in* CD_3OD. δ in ppm, *J* in Hz.

	1		**2**	
	δ(C)[a])	δ(H)[b])	δ(C)[c])	δ(H)[d])
CH_2(1)	23.4	1.99–2.13 (*m*)	27.4	2.64–2.77 (*m*, H_a), 2.05–2.12 (*m*, H_b)
CH_2(2)	30.9	2.31–2.44 (*m*)	21.7	2.26–2.38 (*m*, H_a), 2.08–2.16 (*m*, H_b)
C(3) or CH_2(3)	176.5		68.2	3.40–3.42 (*m*, H_a), 3.37–3.38 (*m*, H_b)
CH_2(5)	35.8	4.03 (*dd*, *J* = 13.3, 5.7, H_β), 3.22 (*dd*, *J* = 13.3, 10.3, H_α)	61.5	3.60 (*ddd*, *J* = 12.1, 4.7, 2.1, H_b), 3.36–3.41 (*m*, H_a)
CH_2(6)	29.3	2.18 (*d*, *J* = 14.6, H_α), 1.74–1.88 (*m*, H_β)	27.4	2.18–2.24 (*m*, H_α), 2.85 (*tdd*, *J* = 13.2, 11.2, 4.7, H_β)
H–C(7)	75.3	4.77 (*d*, *J* = 2.4, H_β)	77.5	4.35–4.46 (*m*, H_α)
H–C(8)	55.4	2.47 (*dd*, *J* = 11.0, 2.4, H_β)	49.6	3.33–3.35 (*m*, H_β)
H–C(9)	54.7	3.83 (*dt*, *J* = 11.0, 6.9, H_α)	73.5	3.42–3.45 (*m*, H_α)
C(10)	195.1		194.5	
C(11)	119.4		120.6	
C(12)	164.2		163.3	
H–C(13)	117.3	6.94 (*d*, *J* = 7.9)	116.7	6.86 (*d*, *J* = 8.1)
H–C(14)	136.6	7.40 (*t*, *J* = 7.9)	136	7.36 (*dd*, *J* = 7.7, 8.1)
H–C(15)	126.3	6.87 (*d*, *J* = 7.9)	125.9	6.84 (*d*, *J* = 7.7)
C(16)	143.8		142.8	
Me(17)	23.3	2.60 (*s*)	22.7	2.56 (*s*)

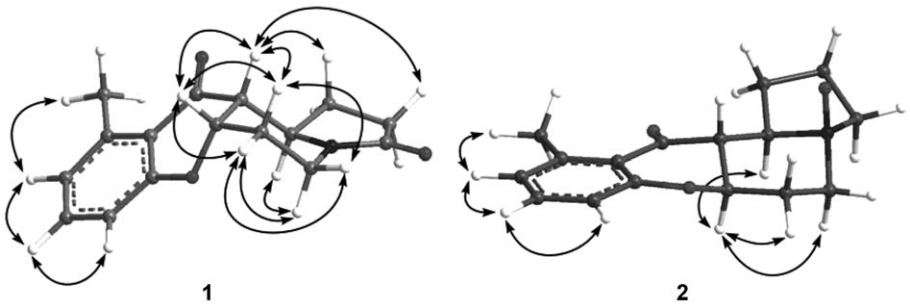
[a]) Recorded at 100 MHz. [b]) Recorded at 400 MHz. [c]) Recorded at 125 MHz. [d]) Recorded at 500 MHz.

In the HMBC spectrum of **1**, correlations from $CH_2(1)$ to C(3), from $CH_2(5)$ and H–C(9) to C(3) and C(7), from H–C(7) to C(9), from H–C(8) to C(1), and from $CH_2(1)$ to C(9) evidenced the structure of the indolizidine moiety and located the additional CO at C(3) correctly (*Fig. 2*). The trisubstituted aromatic system was established from HMBC correlations of Me(17) and H–C(13) to C(15), H–C(14) to C(16) and C(12), and H–C(15) and Me(17) to C(11). The relative configuration of compound **1** was deduced from the vicinal coupling constants (*Table*) and NOESY correlations (*Fig. 3*). The H-atom H–C(7) resonating as a narrow *multiplet* with small coupling constants indicated that H–C(7) was in an equatorial position and assigned β-orientation, similar to that of (±)-isoelaeocarpine (**3**) and unlike that of (±)-elaeocarpine [2d]. The NOESY correlation between H–C(7) and H–C(8), and large coupling constant ($J = 11.0$ Hz) between H–C(8) and H–C(9) revealed that H–C(8) is β-configurated and H–C(9) α-oriented. The specific optical rotation of **1** is about zero, indicating alkaloid **1** is a racemate. The structure of compound **1** was finally determined as (±)-3-oxoisoelaeocarpine.



1 **2**

Fig. 2. *Key HMBCs* (H → C) *of compounds* **1** *and* **2**



1 **2**

Fig. 3. *Optimized 3D structures and key NOESY correlations* (H ⇌ H) *of compounds* **1** *and* **2**

The molecular formula of alkaloid **2** was determined as $C_{16}H_{19}NO_3$ on the basis of HR-ESI-MS data (*m*/*z* 274.1435 ($[M+H]^+$; calc. 274.1438)). The IR and UV spectra also indicated an *Elaeocarpus* alkaloid with an aryl C=O group for **2**. The ^{1}H-NMR spectrum (*Table*) exhibited three aromatic characteristic H-atom signals between δ(H) 6.86 and 7.36, and a sharp Me signal at δ(H) 2.56. These data indicated a structure similar to that of (±)-elaeocarpine (**4**) for compound **2**. Compared to those of (±)-elaeocarpine (**4**) [6e], the ^{13}C-NMR data (with DEPT) showed similar C-atom signals for six aryl C-atoms at δ(C) 163.3, 142.8, 136.0, 125.9, 120.6, and 116.7, one CO C-atom at δ(C) 194.5, and one Me group at δ(C) 22.7. Three strongly deshielded aliphatic C-

atom signals at δ(C) 73.5, 68.2, and 61.5, and an additional O-atom indicated that **2** is the *N*-oxide of (±)-elaeocarpine (**4**).

An HMBC experiment showed the same C-skeleton of **2** as that of (±)-elaeocarpine (**4**). In the HMBC spectrum (*Fig. 2*), correlations of CH_2(3) to C(1) and C(5), CH_2(1) to C(9) and C(8), H–C(9) to C(7), and CH_2(6) to C(8) secured the structure of the indolizidine moiety. The trisubstituted aromatic system moiety was established from the correlations of Me(17) to C(11) and C(15), H–C(14) to C(16) and C(12), and H–C(15) to C(11). These two moieties were connected by correlations from H–C(7) to C(10).

The relative configuration of compound **2** was also deduced from the vicinal coupling constants (*Table*), a NOESY experiment (*Fig. 3*), and previous findings [2d]. A broad *multiplet* at δ(H) 4.35–4.46 for H–C(7) was similar to the signal observed for (±)-elaeocarpine (**4**), which suggested α-orientation for H–C(7) and β-orientation for H–C(8). NOESY Correlation between H–C(7) and H–C(9) indicated an α-orientation for H–C(9). To fully confirm the above conclusion, a chemical transformation of (±)-elaeocarpine (**4**) to its *N*-oxide **2** was accomplished by *m*-chloroperbenzoic acid (*m*-CPBA) oxidation (see *Exper. Part*). Finally, lack of optical activity suggested the structure of alkaloid **2** as (±)-elaeocarpine *N*-oxide.

The racemic mixture of **1** was separated on a *Shiseido* chiral *CD-Ph* column eluting with 70% aqueous MeOH at a flow rate of 0.8 ml/min (*Fig. 4*). Two enantiomers, **1a** and **1b**, were prepared and their ECD spectra were recorded. Simultaneously, a time-dependent density-functional theory (TD-DFT) computational-chemistry study for the two enantiomers was carried out following similar procedures we previously reported [9]. Briefly, conformation searching at MMFF 94 molecular-mechanics force field by using the Omega 2.1 program [10] showed only one dominating conformer for **1a** and **1b**, respectively. The resulting conformations were re-optimized using DFT at the B3LYP/6-311++G (2d, 2p) level using GAUSSIAN 09 software package [11]. The B3LYP/6-311++G (2d, 2p) harmonic vibrational frequencies were further calculated to confirm their stability. A TD-DFT calculation of the ECD data was carried out at the B3LYP/aug-cc-pVDZ level. To obtain the ECD spectrum, the Gaussian 09 calculation results were processed with the Gausssum 2.2 software package ($\sigma = 0.6$) [12]. The calculated ECD spectrum for (7*S*,8*R*,9*R*)-3-oxoisoelaeocarpine showed first negative (348 nm) and second positive (303 nm) *Cotton* effects, similar to those of **1a** (positive at 353 and negative at 318 nm), which allowed the determination of the absolute configuration of **1a** as (7*S*,8*R*,9*R*). Similarly, the structure of **1b** was determined as (7*R*,8*S*,9*S*)-3-oxoisoelaeocarpine. (±)-Isoelaeocarpine (**3**) was also separated on the chiral *CD-Ph* column eluted with 70% aqueous MeOH at a flow rate of 0.5 ml/min to give **3a** and **3b** (*Fig. 4*). Following the same protocols as compound **1**, **3a** and **3b** were determined as (7*S*,8*R*,9*R*)- and (7*R*,8*S*,9*S*)-isoelaeocarpine, respectively (*Fig. 5*).

Experimental Part

General. Column chromatography (CC): commercial silica gel for TLC (SiO_2; *Qing Dao Hai Yang Chemical Group Co., Ltd*); *C18* column (*Phenomenex 00G-4324-N0*; 10 μm, 10 mm (i.d.) × 25 cm); *MCI-GEL* (*MITSUBISHI*, Japan); and chiral *CD-Ph* column (*Shiseido*, Japan). HPLC: *Agilent1100*, American. TLC: precoated silica gel plates (*HSGF 254*, *Yan Tai Jiang You Silica Gel Development Co.,*

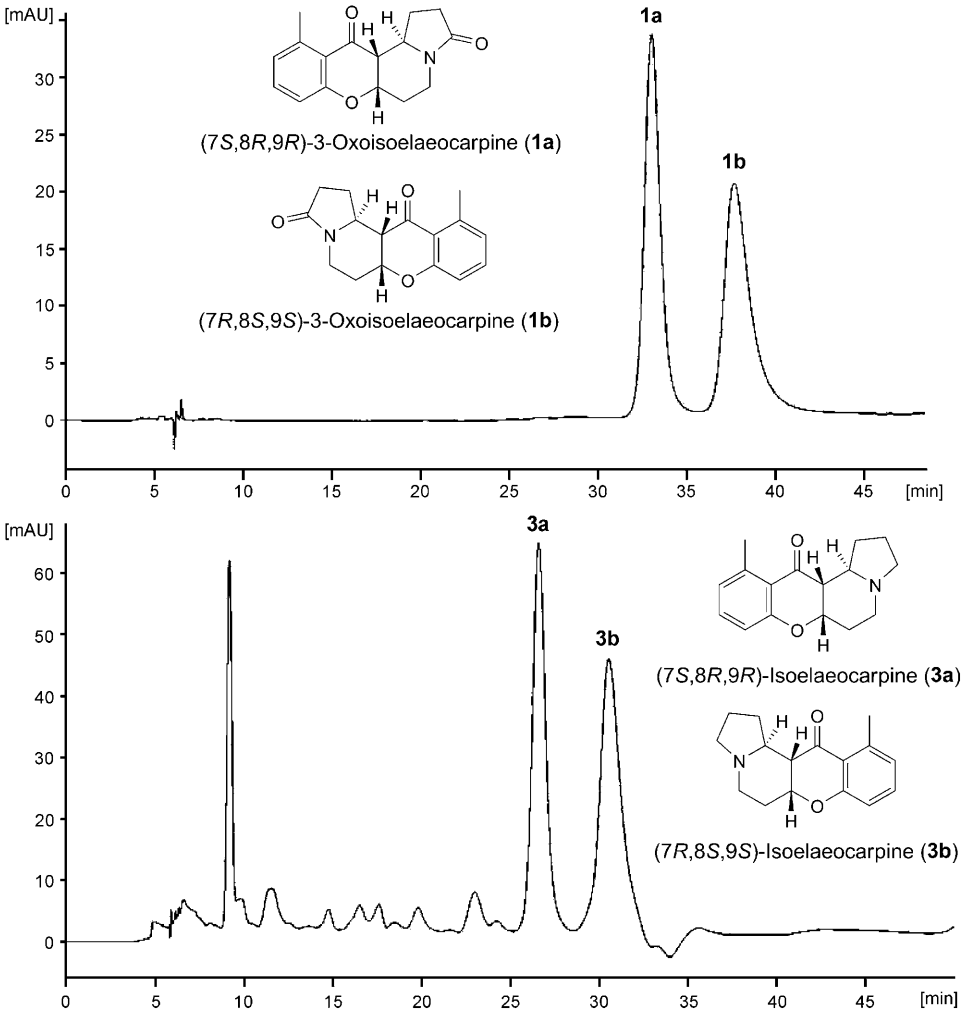


Fig. 4. *HPLC Spectrum for optical resolution of compound* **1** (top) *and* **3** (bottom). Compound **1**: capacity factors, $K_1 = 4.84$, $K_2 = 5.67$; selectivity factor, $\alpha = 1.17$; resolution, $R = 2.27$. Compound **3**: capacity factors, $K_1 = 6.61$, $K_2 = 7.85$; selectivity factor, $\alpha = 1.19$; resolution, $R = 2.34$).

Ltd). Optical rotations: *JASCO P-1010* polarimeter. ECD Spectra: *JASCO J-815* CD spectrometer. UV Spectra: *BECKMAN DU-600* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Bruker VECTOR-22* spectrophotometer; KBr pellets; in cm^{-1}. NMR Spectra: *Varian Unity-INOVA-400/54* spectrometers; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-MS: *Micromass Quattro* triple-quadrupole mass spectrometer equipped with an ESI source (*Micromass*, Manchester, UK); in m/z (rel. %).

Plant Material. The branches and leaves of *Elaeocarpus sphaericus* were collected from Hainan Province, P. R. China, in June 2008 and identified by Prof. *Shi-Man Huang* of Hainan University. A voucher specimen has been deposited with the Institute of Modern Chinese Medicine, Zhejiang University (accession No. ES2008).

Extraction and Isolation. Branches and leaves (10 kg) of *Elaeocarpus sphaericus* were dried, powdered, and then extracted with 95% EtOH at r.t. After removal of the solvent under reduced

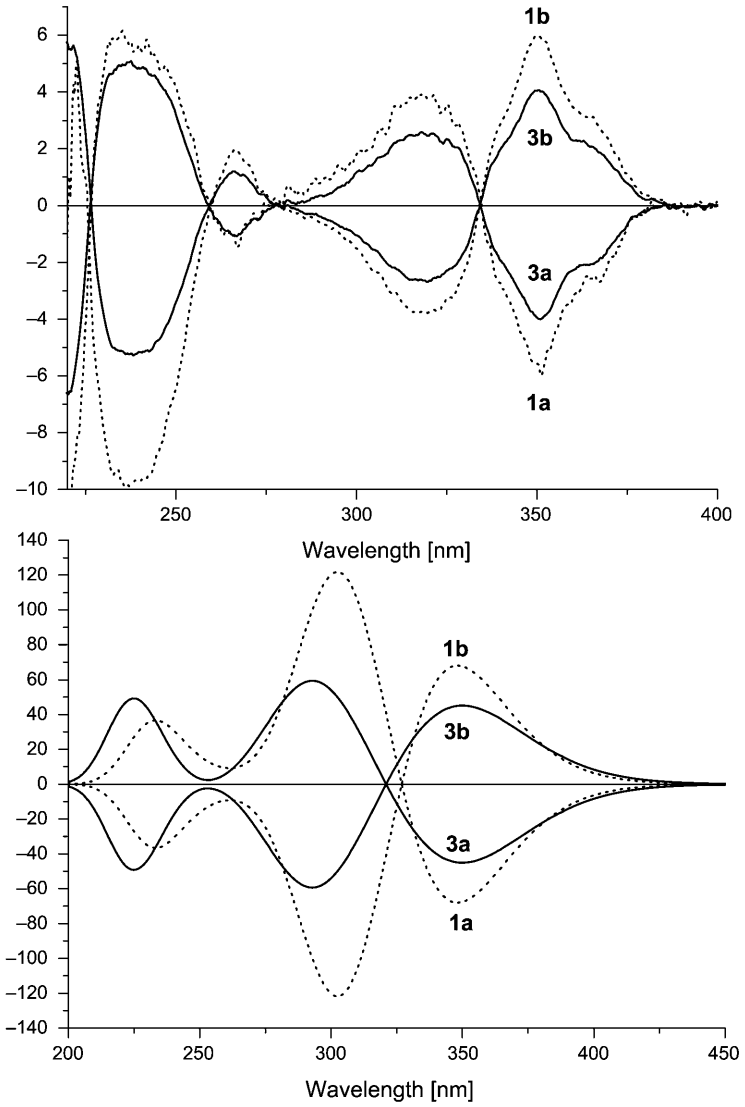


Fig. 5. *Experimental CD spectra* (top) *and TD-DFT-calculated CD spectra* (bottom) *for enantiomers of* **1** (dashed lines) *and* **3** (solid lines)

pressure, the crude extract (283 g) was dissolved in 1 l H_2O and adjusted to pH 3 with 2M HCl, followed by extraction with AcOEt. The aq. layer was then adjusted to pH 10 with Na_2CO_3, and extraction with AcOEt gave a crude alkaloid fraction (45.5 g). The crude alkaloid fraction was then subjected to a *MCI-GEL* column (H_2O/EtOH 100:0–95:5) to give four fractions, *Frs. A–D. Fr. A* was further chromatographed over SiO_2 column ($CHCl_3$/MeOH/Et_2NH 50:1:0.1) to give *Frs. A1* and *A2. Fr. A2* was repurified through a *C18* reversed-phase (RP) column (EtOH/H_2O 1:9) to afford compound **2** (5 mg). *Fr. B* was chromatographed over a SiO_2 column (petroleum ether (PE)/AcOEt 4:1) to yield **4** (100 mg),

and *Fr. B1* and *Fr. B2*. *Fr. B1* was further purified by a *C18* RP column (60% aq. MeOH) to give alkaloid **1** (5 mg). *Fr. B2* was further chromatographed through a SiO_2 column (PE/AcOEt/Et_2NH 15:1:0.1), followed by HPLC purifications to give **3** (MeOH/H_2O 90:10; 14.3 mg) and **5** (MeOH/H_2O 70:30; 4.4 mg).

Preparation of (±)-Elaeocarpine N-*Oxide* (**2**) *from (±)-Elaeocarpine* (**4**). (±)-Elaeocarpine (**4**; 14.8 mg) was dissolved in 8 ml of $CHCl_3$, and then 123.7 mg *m*-CPBA (*m*-chloroperbenzoic acid) in 5 ml of $CHCl_3$ was added in dropwise. The mixture was stirred for 1 h at r.t. After workup, the crude alkaloid was subjected to CC (SiO_2; AcOEt/MeOH 5:1 (0.1% (*v*/*v*) Et_2NH)) to give compound **2** (5 mg).

(±)-3-Oxoisoelaeocarpine (= *(6a*S*,12a*R*,12b*R*)-1,2,6,6a,12a,12b-Hexahydro-11-methyl-5*H*-chromeno[2,3-*g*]indolizine-3,12-dione*; **1**). White amorphous powder. UV (MeOH): 217 (4.25), 258 (3.90), 326 (3.51). IR (KBr): 2968, 2922, 2881, 1683, 1599, 1472, 1422, 793. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS: 272 ($[M+H]^+$). HR-ESI-MS (pos.): 272.1286 ($[M+H]^+$, $C_{16}H_{18}NO_3^+$; calc. 272.1281).

(±)-Elaeocarpine N-*Oxide* (= *(6a*R*,12a*R*,12b*R*)-1,2,3,5,6,6a,12a,12b-Octahydro-11-methyl-12*H*-chromeno[2,3-*g*]indolizin-12-one 4-Oxide*; **2**). White amorphous powder. UV (MeOH): 217 (4.01), 254 (3.66), 322 (3.26). IR (KBr): 3442, 2972, 2820, 1688, 1602, 1470, 792. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS: 274 ($[M+H]^+$). HR-ESI-MS (pos.): 274.1435 ($[M+H]^+$, $C_{16}H_{20}NO_3^+$; calc. 274.1438).

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