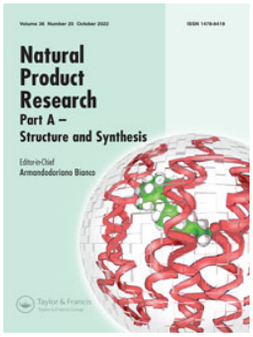

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Rinderidine and oblongifolidine new pyrrolizidine alkaloids from *Rindera oblongifolia* M. Popov and their absolute configurations

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ABSTRACT

The alkaloid composition of *Rindera oblongifolia* was studied, in which the pyrrolizidine alkaloids echinatine and trachelanthamine N-oxide, as well as two new quaternary salts namely rinderidine and the oblongifolidine were isolated. The structures of the isolated new alkaloids were elucidated by NMR spectroscopy. The absolute configuration of lindelofine, trachelanthamine N-oxide, rinderidine and oblongifolidine was established by single crystal X-ray diffraction as: 1*R*, 4*R*, 8*R*, 2′*S*, 3′*R*; 1*R*, 4*S*, 8*S*, 2′*S*, 3′*R*; 4*R*, 7*S*, 8*R*, 2′*S*, 3′*S*; 4*R*, 7*S*, 8*R*, 2′*S*, 3′*S* (7″*S*, 8″*R*) respectively. Both new pyrrolizidine alkaloids showed no cytotoxicity against four cancer cell lines such as HeLa, HEp-2, HBL-100 and CCRF-CEM.

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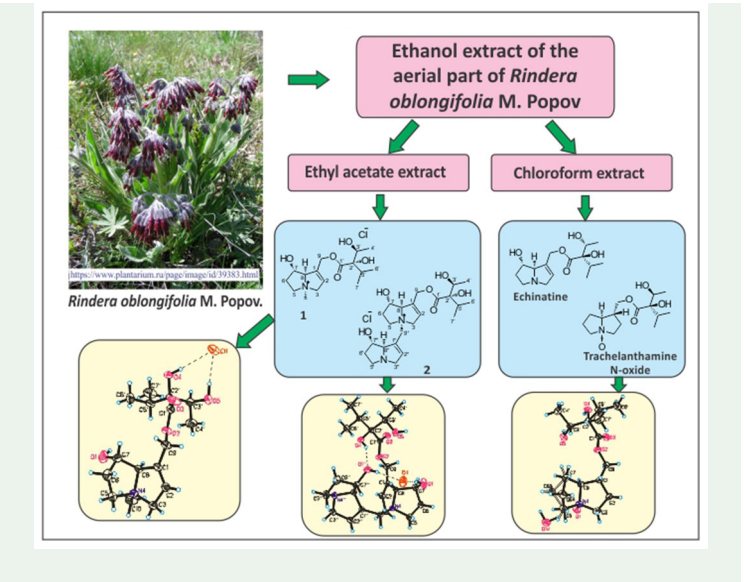

1. Introduction

The family *Boraginaceae* is represented by more 130 genera and approximately 2300 species in the world (Ganos et al. 2020). There are 30 species of *Rindera* belonging to the *Boraginaceae* family worldwide, 17 species grow in the CIS countries, 15 species in Central Asia, and 8 species in Uzbekistan (Shishkin 1953). The plant of *Rindera oblongifolia* M. Popov (family *Boraginaceae*) is widely distributed in Central Asia (Vvedenskiy 1961). In the work of Hilger et al. (2015), the plant was included to the more complex genus *Cynoglossum* with the name *Cynoglossum oblongifolium* (Popov) Greuter & Stier and *R. oblongifolia* was mentioned as a basionym. However, as a result of the critical revision of the type specimens of the *Heliotropiaceae* and *Boraginaceae* taxa names, kept in the National Herbarium of Uzbekistan at the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan (TASH, Tashkent), the name *R. oblongifolia* M. Popov was given as the accepted (Ovchinnikova et al. 2020). Furthermore, the plant name *R. oblongifolia* M. Popov is included in The World Checklist of Vascular Plants database as an accepted name (Govaerts et al. 2021). *R. oblongifolia*, which produces pyrrolizidine alkaloids, is widespread in Uzbekistan (Sadritdinov 1979). The overwhelming majorities of pyrrolizidine alkaloids are substances with high biological activity and are widely used in medicine (Roeder 1995). For example, pyrrolizidines – platyphylline and sarracine have an anticholinergic and antispasmodic effect and are widely used for spasms of smooth muscles of the abdominal organs, bronchial asthma and arterial hypertension. In addition, it was noted that some pyrrolizidine alkaloids exhibit antimitotic, antitumor (trichodesmine, indicine N-oxide), hypotensive (Bull et al. 1968; Sadritdinov 1979), antispasmodic, mydriatic and antiacetylcholinesterase activity (Benamar et al. 2016, 2017, 2021).

Pyrrolizidine alkaloids occur as free necines (either the necine base heliotridine or retronecine) or as a mixture of free bases and their *N*-oxides. They can form single

esters (monoester) at C-9 or C-7, open chain diesters at both C-7 and C-9 of the necine base, or in rare cases macrocyclic diesters linking C-7 with C-9 (El-Shazly and Wink 2014). Until now, the alkaloid composition of *R. oblongifolia* as well as the physicochemical and pharmacological properties of the isolated substances have not been sufficiently studied, although the plant's reserves in the region and the yields of pyrrolizidine alkaloids are sufficient for industrial scale production. In this regard, we have begun to study the alkaloid composition of the aforesaid plant.

We investigated the aerial part of *R. oblongifolia*, collected from the vicinity village of Pskom region of Tashkent. Two new pyrrolizidine alkaloids as quaternary salts named rinderidine (**1**) and oblongifolidine (**2**) as well as two known compounds – echinatine and trachelanthamine N-oxide were isolated. Herein, we describe their isolation and structure elucidation by spectroscopic data as well as absolute configuration of the isolated pyrrolizidine alkaloids by single crystal X-ray diffraction. In addition, the cytotoxic properties of the new compounds were studied *in vitro*.

2. Results and discussion

2.1. Identification of new compounds by NMR data

Compound **1** was obtained as transparent crystals. Its molecular formula was established as $[C_{16}H_{28}NO_5]^+[Cl]^-$ (three degrees of unsaturation) from HPTLC-MS peak at *m/z* 314.1632 $[M\text{-}Cl]^+$ (calcd. for $[C_{16}H_{28}NO_5]^+$, 314.1967). The IR spectrum the compound **1** showed absorption bands at 3419 (OH), 3210 (OH), 3095 (OH) and 1722 (C=O) cm^{-1} indicating the presence of hydroxyl and carbonyl groups.

The structure of the compound **1** was established based on the 1 D and 2 D NMR spectra. The ^1H NMR spectrum was contained characteristic proton signals of 1,2-unsaturated necine by the presence of broadening signals at δ_H 5.90 (H-2) and 4.63 (H-8), two doublet signals with the geminal coupling constant of 16.2 Hz at δ_H 4.36 (H-3a) and 4.44 (H-3b), multiplet signals at δ_H 2.12 (H-6a), 2.19 (H-6b), 3.77 (H-5a), 3.85 (H-5b) and 4.81 (H-9) (El-Shazly and Wink 2014). In addition, three methyl group doublet signals at δ_H 1.20 ($J = 6.6$ Hz, H-4′), 0.87 ($J = 6.8$ Hz, H-6′), 0.79 ($J = 6.8$ Hz, H-7′), quartet signal at δ_H 3.91 ($J = 6.6$ Hz, H-3′) and overlapped multiplet at δ_H 2.12 (H-5′) (Table S1) which characteristic for the (+) viridifloric acid moiety (Logie et al. 1994) were detected in the spectrum of compound **1**. There was observed proton resonance at δ_H 3.33 as a singlet which was assigned to N-CH_3 group.

Analysis of the ^{13}C NMR and DEPT spectra of compound **1** showed the presence of 16 carbon resonance lines represented as four methyl, four methylene, five methine and three quaternary carbons, including one ester carbonyl group at δ_C 174.8 (C-1′). In the aromatic part of the ^{13}C NMR spectrum two signals of the olefinic carbons at δ_C 134.3 (C-1) and 124.5 (C-2) were observed. Eight carbon signals connected to a heteroatom including one methyl group were observed in the range of δ_C 55.0–94.3 ppm. In the highfield region of the spectrum carbon signals of two methylene groups at δ_C 33.8 (C-5′) and 34.2 (C-6) ppm, and three methyl groups at δ_C 16.3 (C-6′), 18.1 (C-4′) and 18.3 (C-7′) were show up (Table S1). Analysis of the ^1H, ^{13}C NMR and DEPT data indicated that **1** had a similar structure to echinatine (Mandić et al. 2013), except one methyl group at δ_C 55.0. The position of the methyl group was

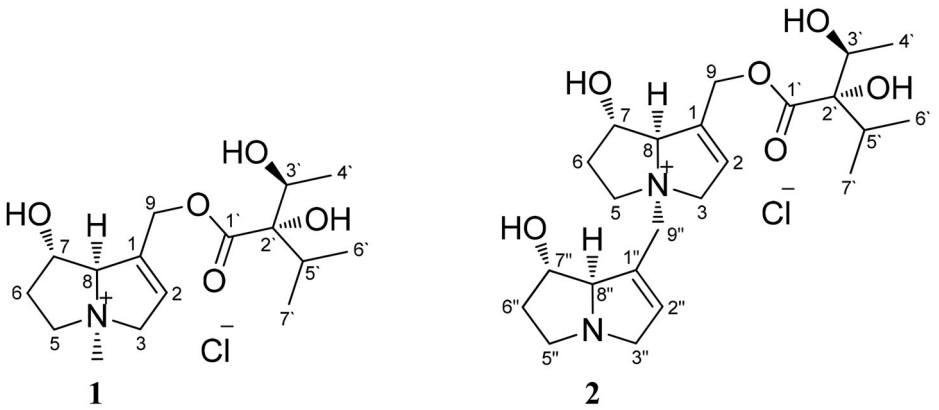

Figure 1. The chemical structures of the pyrrolizidine alkaloids **1** and **2**.

established based on the HMBC cross peaks of CH_3/C-3, C-5, C-8 at the N atom in alkaloid. Furthermore, carefully comparison of ^{13}C NMR spectra of echinatine and **1** was revealed deshielding of the carbon atoms C-3 ($\Delta\delta_C = +12.3$ ppm), C-5 ($\Delta\delta_C = +12.4$ ppm) and C-8 ($\Delta\delta_C = +14.5$ ppm) in **1**. The analysis of chemical shifts differences of carbon atoms demonstrated the quaternary form of N atom and the presence of a chloride anion in **1**. Besides, the presence of chlorine was also confirmed by the modified Beilstein test for halogens (Hayman 1939). Based on these results, we thus proposed the structure **1** shown in Figure 1 and named rinderidine. The results of the ^1H and ^{13}C NMR spectra of the pyrrolizidine alkaloid **1** are given in the Table S1.

Molecular formula of pyrrolizidine alkaloid **2** was established as $[C_{23}H_{37}N_2O_6]^+[Cl]^-$ (six degrees of unsaturation) from HPTLC-MS peak at *m/z* 437.3641 $[M\text{-}Cl]^+$ (Calcd. for $[C_{23}H_{37}N_2O_6]^+$, 437.2652). The IR spectrum the compound **2** showed absorption bands at 3381 (OH), 3240 (OH), 3030 (OH) and 1741 (C=O) cm^{-1} indicating the presence of hydroxyl and carbonyl groups. A comparison of the ^1H and ^{13}C NMR spectroscopic data of compound **2** (Table S1) with those of pyrrolizidine alkaloid **1** indicated that these two compounds were similar in structure. The significant difference in ^1H and ^{13}C NMR spectra of alkaloid **2** was the presence of one additional 1,2-unsaturated necine moiety signals and one methylene group signals (δ_C 64.2, δ_H 4.22, 4.51) as well as disappearing signal of N-CH_3 group. The HMBC correlations between H-9′′ and C-1′′, C-2′′, C-3, C-5 and C-8 indicated that the additional 1,2-unsaturated necine moiety was attached to N atom. The modified Beilstein test for halogens showed the presence of a chlorine in the molecule (Hayman 1939). Detailed ^1H and ^{13}C NMR spectra data of compound **2** are shown in Table S1. Thus, the structure of compound **2** was established as shown in Figure 1 and given a name oblongifolidine.

2.2. X-ray diffraction analysis

To unambiguously establish the absolute configuration of pyrrolizidine alkaloids, a single crystal X-ray diffraction analysis was performed. The structure of molecules **1** and **2** according to X-ray diffraction data is shown in Figure 2. The Flack parameters, which determined the absolute configuration of the molecule, are 0.03 (1) and 0.010 (3) for

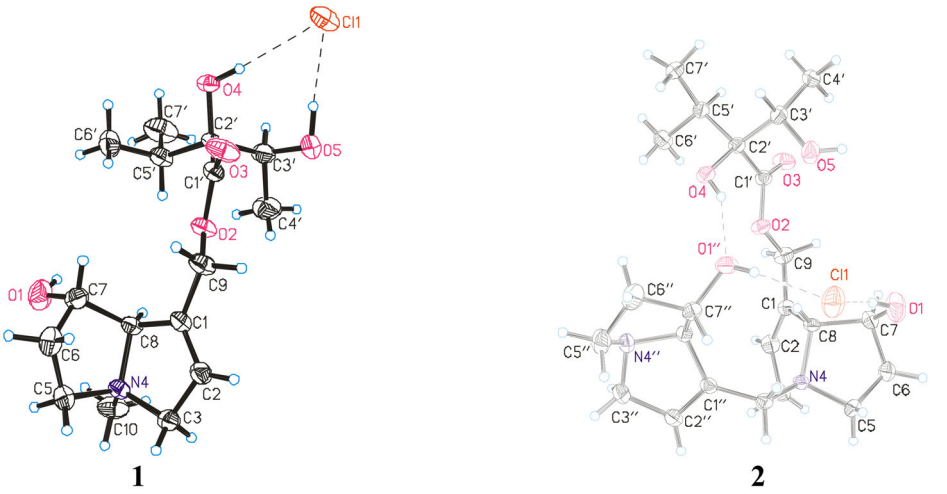

Figure 2. The spatial structure of **1** and **2**, in which are shown intramolecular H-bonds in chlorides.

compounds **1** and **2**, respectively. The absolute configuration of echinatine itself is considered known (Buckingham et al. 2010) and its structure was established by X-ray structural analysis (Gable et al. 1988). The descriptors of chiral centers generally correspond to those found for echinatine, but in molecules **1** and **2**, the nitrogen atom participates as a chiral center. Therefore, the descriptors for chirality at **1** have 4*R*, 7*S*, 8*R*, 2′*S*, 3′*S*. The alkaloid molecule of **2** consists of echinatine and the addition of its alkaloid deoxyheliotridine to the nitrogen at the C-9 position. Therefore, compound **2** has the configuration of chiral centers: 4*R, 7S,* 8*R,* 2′*S,* 3′*S (7"S,* 8"*R)*. In compounds **1** and **2**, the ***A/B***-cycles are *cis*-fused, while the ***B*** cycle is flat everywhere and the ***A*** cycle takes shape between the envelope and the twist.

In crystal structure of **1**, the hydrogen atoms of the OH group of the initial alkaloid (cation) and transformed by the symmetry element 2_1 are directed towards the Cl ion, and form an intermolecular H-bond of the O-H … Cl type. As a result, a chain is formed along the crystallographic axis *a*. The carbonyl oxygen of the acyl group forms an H-bond of the C-H … O type (Table 1).

In the structure of **2**, an H-bond of the O-H … Cl type is formed between the hydrogens of the same OH-groups at C-7 (heliotridine and echinatine parts of 2) of the cation and the Cl^- ion. There is also an intramolecular H-bond between the hydrogen of the OH group at C2′ and the oxygen of the OH group at C-7′. The parameters of these H-bonds are given in Table 1. The intermolecular H-bond between the tetrahedral nitrogen N4 of the initial molecule and the hydrogen of the hydroxyl group at C-3" transformed by the screw axis forms a chain along the crystallographic axis *c* (Table 1).

Base **3** was identified on the basis of high performance thin layer chromatography (HPTLC) data and a sample of mixing with a true sample of echinatine, previously obtained from the *Solenanthus karategenius* and *R. baldshuanica* (Akramov et al. 1958, 1964).

The structure of alkaloid **4** was established by X-ray diffraction as trachelanthamine N-oxide. This alkaloid was isolated from *R. oblongifolia* for the first time by us, which was previously isolated from *Trachelanthus korolkovii* (family Boraginaceae)

Table 1. Hydrogen bonds in the crystal of **1**, **2**, **4** and lindelofine (*D*-donor, *A*-acceptor).

Structures	Bond *D*-H … *A*	Length, (Å) *D*-H	Length, (Å) H … *A*	Length, (Å) *D* … *A*	Angle, (°) *D*-H … *A*	Symmetry
1	O1-H1 … Cl1	0.85 (6)	2.32 (6)	3.122 (4)	158	*x, y, z*
	O4-H4 … Cl1	0.92 (6)	2.35 (6)	3.230 (3)	161	*5/2-x, 1-y, 0.5 + z*
	O5-H5 … Cl1	0.99 (7)	2.18 (7)	3.163 (4)	170	*5/2-x, 1-y, 0.5 + z*
	C10-H10A … O3	0.96	2.57 (2)	3.382 (6)	142	*3/2-x, 1-y, −0.5 + z*
2	O1-H1 … Cl1	1.04 (8)	2.02 (8)	3.060 (4)	170	*x, y, z*
	O1″-H1′ … Cl1	0.80 (4)	2.17 (4)	2.963 (3)	172	*x, y, z*
	O4-H4 … O1″	0.76 (4)	2.08 (4)	2.774 (3)	152	*x, y, z*
	O5-H5 … N4″	0.83 (5)	1.99 (5)	2.817 (3)	172	*y, -x + y, −1/6 + z*
4	O4-H4 … O1	0.72 (3)	2.08 (3)	2.762 (2)	159	*-0.5 + x, 3/2-y, -z*
	O5-H5 … O1	0.85 (3)	1.85 (3)	2.695 (2)	170	*-0.5 + x, 3/2-y, -z*
	O1W-H1 … O1	1.05 (5)	1.75 (5)	2.802 (3)	178	*x, y, z*
	O1W-H2 … O3	1.04 (5)	1.95 (5)	2.967 (3)	163	*-0.5 + x, 3/2-y, -z*
Lindelofine	O4-H4 … N4	0.92 (4)	1.92 (4)	2.759 (3)	152	*1-x, −0.5 + y, 0.5-z*
	O5-H5 … O4	0.81 (5)	2.15 (5)	2.962 (3)	175	*0.5 + x, 3/2-y, 1-z*
	C3-H3A … O3	0.97	2.59 (2)	3.402 (4)	147	*0.5 + x, 3/2-y, -z*
	C8-H8A … O3	0.98	2.48 (2)	3.271 (4)	137	*1-x, 0.5 + y, 0.5-z*

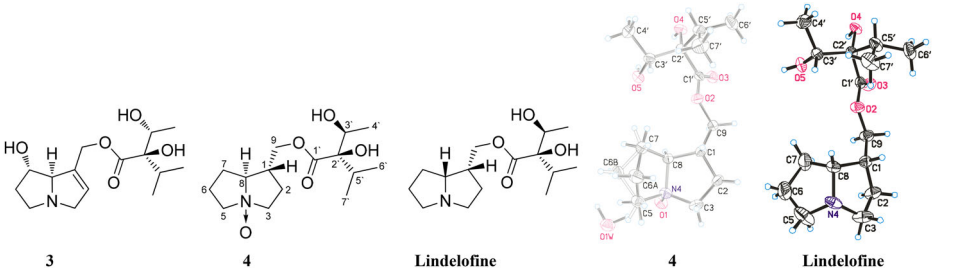

Figure 3. Chemical and spatial structures of alkaloids **3**, **4** and lindelofine.

(Men'shikov and Borodina 1945; Akramov et al. 1967). The alkaloids trachelanthamine and lindelofine (Abdullaev et al. 1972) are 8-epimers and their absolute configuration was proposed on the basis of spectral data and is given in Buckingham et al. (2010). Repeated isolation of trachelanthamine in the form of N-oxide (**4**) suggested to performing an additional X-ray analysis of lindelofine in order to independently and reliably establish as well as comparing the absolute configuration of the chiral centers of these alkaloids.

The spatial structure of alkaloids N-oxide of trachelanthamine (**4**) and lindelofine (from the laboratory of alkaloids of Institute of the Chemistry of Plant Substances and isolated from *R. cyclodonta* (Abdullaev et al. 1972)) according to X-ray diffraction data is shown in Figure 3. The performed X-ray diffraction experiment makes it possible to establish the absolute configuration of lindelofine and **4**, the Fleck parameters are 0.14 (8) and 0.02 (9), respectively. Although for the latter, the absolute configuration is established in the form of trachelanthamine monohydrate N-oxide. Chiral centers, including the N-4 nitrogen atom, have the following descriptor values: 1 *R*, 4 *R*, 8 *R*, 2′*S*, 3′*R* and 1 *R*, 4*S*, 8*S*, 2′*S*, 3′*R* respectively, which confirms the configuration proposed in the literature (Buckingham et al. 2010).

In crystals of alkaloids **4** and lindelofine, the mutual arrangement of active functional groups indicates the absence of intramolecular H-bonds. In alkaloids of

lindelofine and **4**, the connection of the ***A/B***-rings was *cis*, although the alkaloids are epimers at the chiral center of C-8 (and easily inverted by nitrogen N-4). In crystal of **4**, disordering of the C-6 atom is observed, which is located in two positions (C-6A and C-6B) with an equal probability. In molecule of **4**, the 6α- and 6β-envelope forms are realised simultaneously in the crystal. In this case, all five-membered cycles in both molecules retain the 2,6-envelope form (Figure 3).

Crystal of **4** was a monohydrate. The H atoms of the crystallization water molecule are hydrogen bonded by the oxygen of the N→O group and the carbonyl oxygen of the acyl group of molecule of **4** (Table 1). Hydrogen atoms of hydroxyl groups are involved in intermolecular H-bonds; they are also directed to the oxygen of the N→O group (Table 1). In the crystal of the lindelofine molecule, an intermolecular H-bond of the O-H … N and O-H … O type with the participation of hydroxyl groups is observed, and the carbonyl oxygen of the acyl group forms an H-bond of the C-H … O type. The parameters of these hydrogen bonds are given in Table 1.

2.3. Biological activity of the new alkaloids

We studied for the first time the cytotoxic activity of new pyrrolizidine alkaloids **1** and **2** against four cancer cell lines such as HeLa, HEp-2, HBL-100 and CCRF-CEM. Both new pyrrolizidine alkaloids showed no cytotoxicity against these cancer cell lines – values of IC_{50}>100 μM.

3. Experimental

3.1. General experimental procedures

The IR spectrum was recorded on a Perkin-Elmer System model 2000 (KBr) Fourier spectrometer. NMR spectra were recorded on a JNM-ECZ400R spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) in CD_3OD. TMS (δ 0.00 ppm) was used as an internal standard for ^{1}H NMR shifts, and solvent signal (CD_3OD, 49.00 ppm vs. TMS) was used as a reference for ^{13}C NMR shifts. NMR spectra were processed using the MestReNova 14.2.0 software (Mestrelab Research S.L., Santiago de Compostela, Spain).

HPTLC chromatographic plates (silica gel 60 F254 (Merck, Germany), alternatively flexible plates TLC AL Sil G/UV) were used for chromatography. Chloroform – methanol solvent system in a ratio of 3:1 was used as an eluting mixture. Elution was carried out in a darkened glass chamber (distance 7 cm). After elution, the plates were dried in air for 10 min, visualised by iodine vapor and UV irradiation (UV lamp, 254 nm) or by spraying with a Dragendorff reagent.

3.2. Plant materials

The aerial part of *Rindera oblongifolia* M. Popov, was collected from the vicinity village of Pskom region of Tashkent. The species were identified by Dr. Nigmatullaev A., an employee of the Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan. A voucher specimen (No 2008) was deposited in the Institute of the Chemistry of Plant Substances.

3.3. Extraction and isolation of compounds

Air dry crushed aerial part of the *R. oblongifolia* (500 g) was extracted with 80% ethyl alcohol. The extraction process was carried out in multifunctional ultrasonic extraction device Delta with stirring at 40 °C for 1 hour. The extraction process was repeated 6 times. The completeness of the extraction of alkaloids was determined by the of silicotungstic acid reagent. The aqueous residue was acidified with 10% H_2SO_4 solution (pH 1-2). The acidic solution was extracted with chloroform. The aqueous solution was alkalised with 25% ammonia (pH 11). Then the alkalised solution was extracted with chloroform (3 × 250 mL) and ethyl acetate (3 × 250 mL). Combined chloroform and ethyl acetate extracts were concentrated on a rotary evaporator to a thick resin. As a result, 1.5 g (0.3% by weight of dry raw material) of chloroform fraction and 2.5 g (0.5% by weight of dry raw material) of ethyl acetate fraction were obtained. Separation of the chloroform sum of alkaloids was carried out by column chromatography with silica gel (90-125 μm, 1:30). The alkaloids were eluted using gradient elution with increasing chloroform:methanol ratio (100:1→100:20) to give 125 fractions. Fractions 101-125 was treated with methanol to obtain crystals of **3** (132 mg). Individual compound of **4** was precipitated from fraction 26–50 of the chloroform extract. Single crystals of the alkaloid **4** were grown from methanol for X-ray diffraction analysis.

An ethyl acetate extract (2.5 g) was chromatographed on a column of KSK grade silica gel (90-125 μm) (1:30) using a gradient elution with an increasing ratio of chloroform:methanol 50:1→15:1) to obtain 250 fractions. After treatment of fractions 87-151 of ethyl acetate extract by ethanol was isolated alkaloid **1** (55 mg, 2.2% by weight of the sum of alkaloids). Single crystals of **1** were grown from ethanol. The separation of fractions 152-247 of the ethyl acetate extract on column chromatography with silica gel by elution chloroform-methanol in gradient polarity of the mixture (7:1–4:1) was isolated alkaloid **2** (112 mg, 4.48% by weight of the sum of alkaloids). Single crystals of **2** were grown from ethanol for X-ray diffraction analysis.

Rinderidine (1) – transparent crystals, $T_{m.p.}$ = 178-179 °C. IR (KBr, ν_{max}, cm^{-1}): 3419 (OH), 3210 (OH), 3095 (OH), 2969, 2919, 2809, 1722 (C=O), 1458, 1388, 1226, 1157, 1119, 1072, 1028, 981, 942, 883, 805, 769, 530. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) are reported in Table S1.

Oblongifolidine (2) – transparent crystals, $T_{m.p.}$ = 202-205 °C. IR (KBr, ν_{max}, cm^{-1}): 3381 (OH), 3240 (OH), 3030, 2972, 2941, 2918, 1741 (C=O), 1459, 1383, 1284, 1225, 1182, 1144, 1111, 1027, 986, 910, 886, 826, 785, 618, 511. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) are reported in Table S1.

3.4. X-ray analysis

The unit cell parameters for single crystals were determined and refined on a CCD Xcalibur Ruby diffractometer (Oxford Diffraction) using CuKα radiation (T = 291 K) (CrysAlisPro 2009). Table S2 shows the main parameters of the X-ray diffraction experiment and calculations. The absorption correction was introduced using the SADABS program (Sheldrick 1996).

The structures were solved by direct methods using the SHELXS-97 software package (Sheldrick 2008). The calculations for the refinement of structures were performed using the SHELXL-2014/8 program (Sheldrick 2015). All non-hydrogen atoms were refined by the least squares method (according to F^2) in the full-matrix anisotropic approximation. The FVAR instruction was used to refine the disordering of the C6A and C6B atoms in structure **4**. Hydrogen atoms at carbon atoms were specified geometrically and refined according to the riding scheme with fixed isotropic displacement parameters $U_{iso} = nU_{eq}$, where $n = 1.5$ for methyl groups and 1.2 for the other (U_{eq} is the equivalent isotropic displacement parameter of the corresponding carbon atoms. Hydrogen atoms of hydroxyl groups and water were revealed from difference electron density (ED) syntheses and refined isotropically. X-ray diffraction analysis materials have been deposited at the Cambridge Crystallographic Data Center (CCDC) in the form of a CIF file.

3.5. Cytotoxic activity assays

HeLa cervical epithelial carcinoma, breast adenocarcinoma HBL-100 (ATCC NTV 124) and adenocarcinoma of the larynx HEp-2 (ATCC:CCL-23) were obtained from the Central Bank of the Cell Culture Collection of the Institute of Cytology, Russian Academy of Sciences, Russian Federation, T-lymphoblastic leukemia CCRF-CEM (ATCC: CCL-19) from Heidelberg University, Germany. Cells were cultured in RPMI-1640 and DMEM medium (Capricorn Scientific, Germany) containing 1% antimycotic antibiotic, 2 mM L-glutamine, 10% FBS (Himedia, India) in a CO_2 incubator (SHELLAB, USA). Compounds were dissolved in DMSO immediately before the experiment.

The cytotoxic properties of the compounds were determined by the *in vitro* MTT method (Niks and Otto 1990). Cells with a solvent served as a negative control, and the "Cysplatin-Naprod" (India) cytostatic agent was used as a positive control. Analysis and statistical processing of the obtained data was carried out using the Origin 8.6 program using the well-known methods of variation statistics with an assessment of the significance of indicators ($M \pm m$) and differences in the considered samples according to Student's t-test. The results were considered significant at $p \leq 0.05$.

4. Conclusions

Thus, the pyrrolizidine alkaloids echinatine and trachelanthamine N-oxide, as well as two new quaternary salts rinderidine and oblongifolidine were isolated from the *R. oblongifolia*. The absolute configuration of lindelofine, trachelanthamine N-oxide, rinderidine, oblongifolidine was established as: 1*R*, 4*R*, 8*R*, 2′*S*, 3′*R*; 1*R*, 4*S*, 8*S*, 2′*S*, 3′*R*; 4*R*, 7*S*, 8*R*, 2′*S*, 3′*S*; 4*R*, 7*S*, 8*R*, 2′*S*, 3′*S* (7′′*S*, 8′′*R*) by single-crystal X-ray diffraction analysis, respectively. New pyrrolizidine alkaloids **1** and **2** showed no cytotoxicity against cancer cell lines HeLa, HEp-2, HBL-100 and CCRF-CEM.

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