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Berberine and coptisine free bases

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Abstract

The free bases of protoberberine alkaloids berberine and coptisine and related compounds have been examined. The ^{1}H and ^{13}C NMR data of 8-hydroxy-7,8-dihydroberberine (**2a**), 8-hydroxy-7,8-dihydrocoptisine (**2b**), bis(7,8-dihydroberberin-8-yl) ether (**3a**), 8-oxoberberine (**5a**), and 8-oxocoptisine (**5b**) as well as X-ray data of compounds **2a**, **5a**, and **5b** are reported and discussed.

Keywords: Berberine; Coptisine; Protoberberine alkaloids; NMR spectroscopy; X-ray crystallography

1. Introduction

Berberine (**1a**) and coptisine (**1b**) are quaternary protoberberine alkaloids. In the form of salts **1** these alkaloids are brightly colored and contribute to tissue coloring of many species of the Berberidaceae, Papaveraceae, and other plant families [1]. Both alkaloids display a number of biological activities and are of current medical interest [2–4]. They are characterized by the sensitivity to nucleophilic attack and several 8-substituted adducts have been described, e.g. with cyanide [5], methoxide [6], chloroform [7], liquid ammonia [8]. In alkaline environment, like other typical alkaloids [9], the salts of berberine (**1a**) and coptisine (**1b**) are converted to their free bases. Although the structures of iminium cations **1** are well known the structures of free bases have not been systematically investigated in detail. Continuing our research on free bases of quaternary isoquinoline alkaloids [10–12] we report here a structural study on berberine and coptisine free bases and closely related compounds (Fig. 1).

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2. Experimental

2.1. General procedures

Melting points were determined on a Mettler FP51 apparatus and are uncorrected. IR spectra were recorded on an ATI Mattson Genesis FTIR spectrophotometer. Mass spectra were taken with a Micromass Q-TOF-2 spectrometer using electrospray (HRESIMS, Accurate Mass Spectra) and a Fisons Trio 1000 quadrupole spectrometer using electron ionization (70 eV, EIMS).

2D NMR spectra were recorded with Bruker Avance DRX-400 and DRX-500 spectrometers operating at frequencies of 400.13 (^{1}H), 100.62 MHz (^{13}C) and 500.13 (^{1}H), 125.76 MHz (^{13}C), respectively. The measurement temperature was 303 K. Solutions were prepared by dissolving compounds (5–80 mg) in 500–700 μl of dimethylsulfoxide-d_6, dichloromethane-d_2, and chloroform-d. TMS was used as an internal standard for ^{1}H and ^{13}C spectra. For detailed experimental setup see Refs. [10,13].

Diffraction data were collected on a Kuma KM-4 four-circle single crystal diffractometer using either ω–2θ scan (**5a**) or ω scan mode (**2a**, **5b**), and corrected for Lorentz and

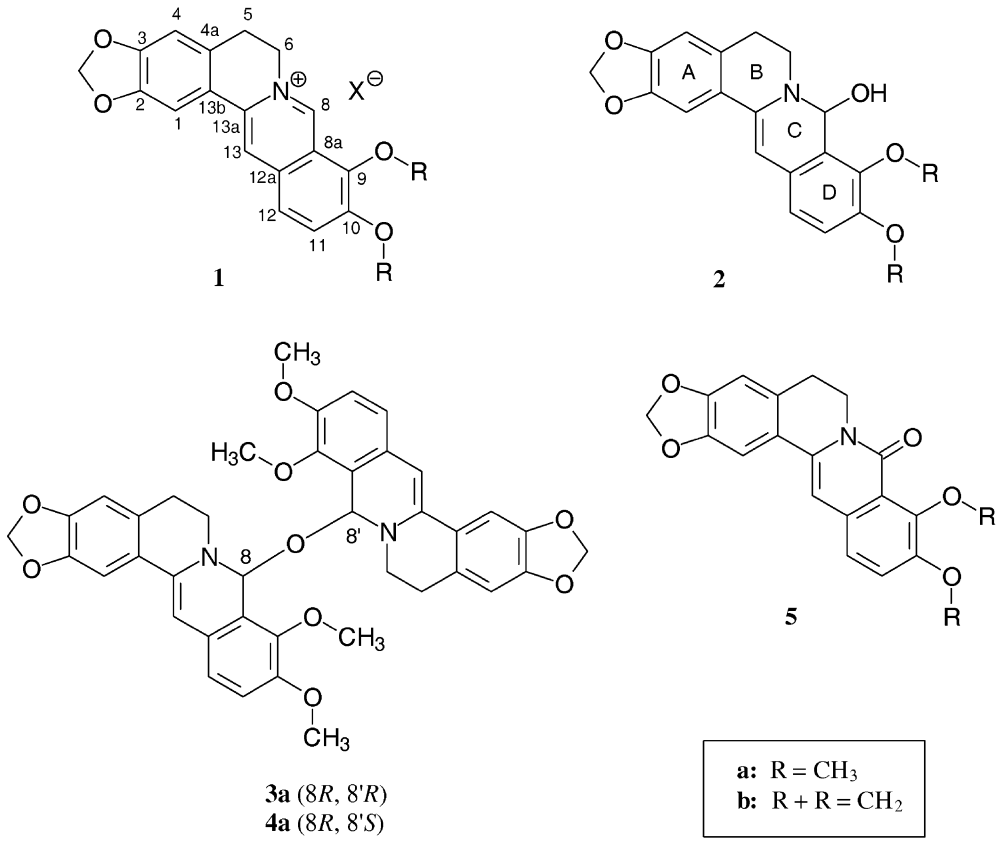

Fig. 1. Structural formulas with atom numbering.

polarisation effects. Structure solution and refinement was done by using a SHELXTL program package [14]. Atomic coordinates and geometric parameters of compounds **2a**, **5a**, and **5b** have been deposited at the Cambridge Crystallographic Data Centre (CCDC), Cambridge, UK (CCDC Ref. nos. are in Table 3).

Berberine chloride (**1a**, X = Cl), berberine hydrogensulfate (**1a**, X = HSO_4), and coptisine chloride (**1b**, X = Cl), all of plant origin, were kindly donated by Professor Jiří Slavík (Masaryk University, Brno, Czech Republic). Spectral characteristics of alkaloids **1** were consistent with their structures.

2.2. Preparation of compounds

2.2.1. 8-Hydroxy-7,8-dihydroberberine (*2a*)

(A) Berberine hydrogensulfate (**1a**, X = HSO_4, 100 mg) was dissolved in water (20 ml) and the aqueous solution was overlayered with diethyl ether (40 ml). A saturated aqueous solution of NaOH was added, the mixture was shaken gently and left at ambient temperature to evaporate. After 2 days, a few light yellow crystals of **2a** were collected. Crystals suitable for X-ray analysis were separated under microscope. M.p. 144–149 °C. IR spectrum (KBr): 3444 (OH), 2935, 1603, 1495, 1382, 1367, 1230, 1169, 1092, 1038 cm^{-1}. EIMS *m/z* 353 (M^+, 3), 352 (17), 351 (51), 337 (16), 336 (M^+–OH, 100), 322 (50), 307 (26), 292 (23). X-ray analysis: Figs. 3 and 4; Tables 3 and 4.

(B) Berberine chloride (**1a**, X = Cl, 5 mg) was dissolved in DMSO-d_6 (550 μl) and 10% NaOH in H_2O (100 μl) was added. The mixture was shaken thoroughly and immediately

Table 1
^{1}H NMR chemical shifts (ppm) of the compounds **2**–**5** in DMSO-d_6

Atom	**2a**	**2b**	**2a**[a,b]	**3a**[a]	**4a**[a]	**5a**	**5b**
1	7.33	7.25	7.15	7.05	7.13	7.45	7.46
4	6.84	6.76	6.62	6.47	6.60	6.89	6.91
5	2.90	2.87	2.86, 2.89	Nd	Nd	2.88	2.87
6	3.45	3.30	3.51, 3.72	Nd	Nd	4.12	4.09
8	6.09	5.96	6.18	6.56	6.40	–	–
11	7.01	6.76	6.94	Nd	Nd	7.49	7.34
12	6.91	6.58	6.90	Nd	Nd	7.39	7.19
13	6.17	6.07	6.08	6.00	6.08	7.04	7.10
2-OCH_2O	6.06	5.99	5.93, 5.95	5.88	Nd	6.05	6.06
9-OCH_2O	–	5.99, 6.02	–	–	–	–	6.19
9-OCH_3	3.87	–	3.91	4.05	4.00	3.79	–
10-OCH_3	3.86	–	3.85	3.91	3.97	3.87	–
8-OH	Nd	Nd	Nd	–	–	–	–

Nd, not observed or assigned.
[a] In CD_2Cl_2.
[b] Data from Ref. [12].

Table 2
^{13}C NMR chemical shifts (ppm) of the compounds **2**–**5** in DMSO-d_6

Atom	**2a**	**2b**	**2a**[a,b]	**3a**[a]	**4a**[a]	**5a**	**5b**
1	104.88	104.88	104.96	104.91	104.89	104.62	104.69
2	147.12	147.08	147.29	Nd	Nd	146.91	146.92
3	147.51	147.46	147.82	Nd	Nd	147.96	147.97
4	108.70	108.67	108.16	107.93	108.20	107.83	107.86
4a	129.97	130.08	129.73	129.50	Nd	129.85	129.84
5	30.57	30.65	30.48	Nd	Nd	27.69	27.61
6	46.42	46.09	46.66	Nd	Nd	38.77	38.63
8	78.69	79.35	79.50	82.80	80.90	158.70	158.18
8a	124.89	114.96	123.46	122.57	122.29	118.50	109.82
9	145.40	143.39	146.17	145.97	143.53	148.32	146.07
10	149.85	144.57	150.33	150.04	150.89	150.86	145.66
11	113.79	108.30	114.58	Nd	Nd	119.11	113.95
12	119.71	116.57	119.72	Nd	Nd	122.52	119.36
12a	128.07	129.02	127.68	128.44	128.74	131.80	131.60
13	95.24	95.09	95.34	96.19	96.98	100.81	101.57
13a	137.23	137.44	136.72	137.22	136.94	134.96	134.83
13b	126.51	126.76	126.21	126.21	Nd	123.12	123.14
2-OCH_2O	101.71	101.69	100.99	100.82	Nd	101.31	101.33
9-OCH_2O	–	101.33	–	–	–	–	102.07
9-OCH_3	61.45	–	61.01	61.14	61.14	60.76	–
10-OCH_3	56.90	–	56.07	56.29	56.34	56.34	–

Nd, not observed or assigned.
[a] In CD_2Cl_2.
[b] Data from Ref. [12].

measured. ^{1}H and ^{13}C chemical shifts of **2a** are in Tables 1 and 2, respectively.

*2.2.2. 8-Hydroxy-7,8-dihydrocoptisine (**2b**)*

(A) Coptisine chloride (**1b**, X = Cl) was treated like **1a** in the preparation of **2a**. A few light yellow crystals of **2b** were obtained. M.p. not observed (decomp. >240 °C). IR spectrum (KBr): 3430 (OH), 2891, 1605, 1481, 1385, 1275, 1230, 1170, 1040 cm^{-1}. EIMS *m/z* 337 (M^+, 3), 336 (12), 335 (47), 321 (76), 320 (M^+–OH, 100), 292 (10).

(B) Coptisine chloride (**1b**, X = Cl, 5 mg) was dissolved in DMSO-d_6 (550 μl) and 10% NaOH in H_2O (100 μl) was added. The mixture was shaken thoroughly and immediately measured. ^{1}H and ^{13}C chemical shifts of **2b** are in Tables 1 and 2, respectively.

*2.2.3. Bis(7,8-dihydroberberin-8-yl) ether (**3a** and **4a**)*

Berberine chloride (**1a**, X = Cl, 80 mg) was suspended in dichloromethane-d_2 (1 ml) and 20% NaOH in H_2O (100 μl) was added, the organic phase was separated, dried with Na_2CO_3, and ^{1}H and ^{13}C NMR spectra were measured. In addition to signals of **2a**, the compounds **3a** and **4a** were detected and their NMR signals were partially assigned (Tables 1 and 2).

*2.2.4. 8-Oxoberberine (**5a**)*

Berberine hydrogensulfate (**1a**, X = HSO_4, 200 mg) was dissolved in water and 30% NaOH in H_2O (4 ml) was added. The mixture was refluxed for 3 h. A precipitate of **5a** was collected and treated with hot 3% hydrochloric acid. Crystallization from ethanol afforded 33 mg of light yellow crystals of **5a**. M.p. 201–203 °C. IR spectrum (KBr): 2929, 1645 (C=O), 1618, 1599, 1490, 1385, 1275, 1225, 1176, 1100, 1095 cm^{-1}. ^{1}H and ^{13}C NMR data: Tables 1 and 2. HRESIMS *m/z* 352.1176 (calcd. for $C_{20}H_{18}NO_5$, 352.1185). Slow recrystallization of **5a** from isopropanol afforded crystals suitable for X-ray analysis (Fig. 5, Table 3).

*2.2.5. 8-Oxocoptisine (**5b**)*

Following NMR measurements of the compound **2b** the DMSO-d_6 solution of **2b** was left at ambient temperature. After 6 months light yellow crystals emerged on the NMR tube walls and were identified as oxocoptisine (**5b**). M.p. 283–285 °C. X-ray analysis: Fig. 6, Table 3. ^{1}H and ^{13}C NMR data: Tables 1 and 2. HRESIMS *m/z* 336.0871 (calcd. for $C_{19}H_{14}NO_5$, 336.0872).

3. Results and discussion

The formation of protoberberine free bases requires a much higher pH value (>12) compared to other isoquinoline alkaloids. This fact is advantageously used for the effective separation of quaternary protoberberines from alkaloid mixtures [15]. The p*K* values of methoxide adducts formation for berberine and coptisine are 15.4 and 13.8, respectively [16]. The free bases of **1** are primarily 8-hydroxyadducts **2**, sometimes called heterocyclic pseudobases [17]. The compounds **2** are rather unstable species sensitive both to acids (which convert them immediately to salts **1**) and to nucleophilic reagents (which may form other types of adducts). The EI-mass spectra of both compounds **2** showed molecular ions of weak intensities and base peaks corresponding to iminium cations **1** (M^+–OH).

3.1. NMR studies

The ^{1}H and ^{13}C NMR spectra of quaternary berberine salt **1a** were analyzed and unambiguously assigned by Blasko et al. [18]. The ^{1}H and ^{13}C NMR chemical shifts of 8-hydroxyadducts **2a** and **2b** in DMSO-d_6 are listed in Tables 1 and 2, respectively. The strategy of signal assignment was analogous to that reported previously [10–12].

In the NMR spectra of **2a** and **2b**, the signals of H-8 (C-8) atoms at 6.09 (78.69) and 5.96 (79.35) ppm, respectively, are of the highest information value. They are significant indicators of hemiaminoacetal structures similarly as it was proved in pseudobases of benzophenanthridine alkaloids [10]. However, the intensities of both H-8 and C-8 signals decreased during time until the peaks disappeared completely. The explanation of this phenomenon may be in consecutive replacement of the H-8 atom with deuterium. As a consequence the C-8 signal was split to a triplet. The signals of OH groups have not been observed in the ^{1}H spectra probably due to the fast chemical exchange in DMSO-d_6 solution.

Table 3
Crystal data and refinement parameters of compounds **2a**, **5a**, and **5b**

Parameter	**2a**	**5a**	**5b**
CCDC reference no.	213767	213769	213768
Empirical formula	$C_{20}H_{19}NO_5$	$C_{20}H_{17}NO_5$	$C_{19}H_{13}NO_5$
Formula weight	353.36	351.35	335.30
Temperature (K)	150(2)	150(2)	120(2)
Wavelength (Å)	0.71073	0.71073	0.71073
Crystal system	Monoclinic	Triclinic	Triclinic
Space group	*C*2/*c*	*P*-1	*P*-1
a (Å)	15.225(2)	7.265(2)	7.282(1)
b (Å)	16.950(2)	14.751(2)	10.107(2)
c (Å)	26.270(3)	15.752(3)	10.567(2)
α (deg)	90	109.80(1)	113.24(3)
β (deg)	98.86(2)	92.57(2)	102.07(3)
γ (deg)	90	101.10(2)	93.97(3)
Volume (Å^3)	6698(1)	1547.5(6)	688.8(2)
Z	16	4	2
Density calculated (Mg/m^3)	1.402	1.508	1.617
Absorption coefficient (mm^{-1})	0.101	0.109	0.119
F(000)	2976	736	348
Crystal size (mm)	0.4 × 0.4 × 0.1	0.5 × 0.3 × 0.2	0.3 × 0.3 × 0.2
Crystal colour	Light-yellow	Light-yellow	Light-yellow
θ Range (deg)	3.33–25.00	2.38–25.00	3.70–25.00
Index range, *h*	−18 → 18	−8 → 8	−8 → 8
Index range, *k*	−20 → 14	−16 → 16	−12 → 12
Index range, *l*	−31 → 31	−18 → 0	−12 → 6
Reflections collected/unique	22630/5883	5443/5253	3889/2347
Data/restraints/parameters	5883/0/476	5253/0/473	2347/0/279
GOF	1.140	1.020	1.129
Final R/wR^2 ($I > 2\sigma/I$)	0.0891/0.2073	0.0337/0.0933	0.0331/0.0939
Final R/wR^2 (all data)	0.1465/0.2665	0.0727/0.1095	0.0381/0.0969

Protoberberine pseudobases **2** are unstable in solution, during a short time (about 12 h) they undergo disproportionation and other products are formed, predominantly 8-oxoderivatives **5**. In the case of coptisine, 8-hydroxydihydrocoptisine (**2b**) and 8-oxocoptisine (**5b**) were present in solution immediately after alkalization of coptisine chloride (**1b**) in DMSO-d_6. The content of oxocoptisine (**5b**) then rapidly increased on account of the compound **2b**. The ^{1}H and ^{13}C chemical shifts of **5a** and **5b** are gathered in Tables 1 and 2. Oxoberberine (**5a**) was therefore prepared synthetically in order to get unambiguous NMR assignment. Oxoderivatives **5** are stable six-membered lactams, resistant to acids. The disproportionation of heterocyclic pseudobases seems to be a general feature observed also in other systems [17,19,20]. Therefore, oxoberberine (**5a**) and oxocoptisine (**5b**) isolated from plants [21,22] are very probably artifacts rather than genuine alkaloids.

The investigation of berberine free base in CD_2Cl_2 revealed some new facts. The organic phase contained not only pseudobase **2a** but also two minor components which were identified by long-range ^{1}H–^{13}C chemical shift correlation experiments [23,24] as diastereomers of bimolecular bis(7,8-dihydroberberin-8-yl) ether **3a** (8*R*,8′*R* + 8*S*,8′*S*, racemate) and **4a** (8*R*,8′*S* = 8*S*,8′*R*, *meso*-form). The ratio of the three components was roughly 10:2:1 (**2a**:**3a**:**4a**). This is a clear analogy with bimolecular aminoacetals in quaternary benzophenanthridine alkaloids [11,20,23–26]. Due to anisotropic effects the signals of H-8 atoms are in more deshielded region at 6.56 (**3a**) and 6.40 ppm (**4a**) compared with the H-8 signals in **2a**. Quantum chemistry calculations for analogical derivative

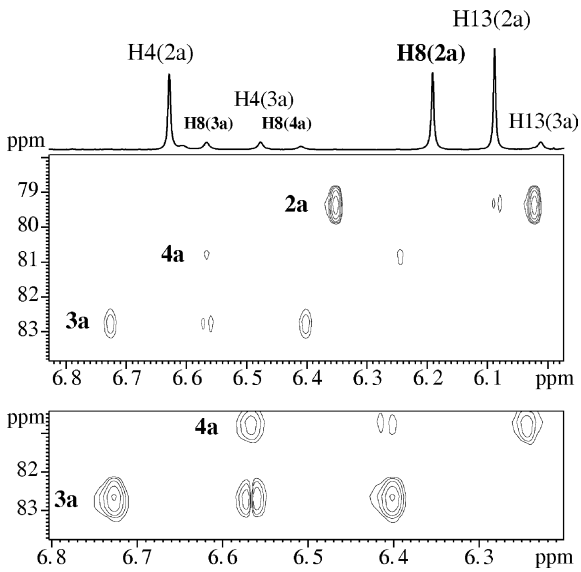

Fig. 2. A portion of GSQMBC spectrum of compounds **2a**, **3a**, and **4a** (top) and a detail of **3a** and **4a** (bottom).

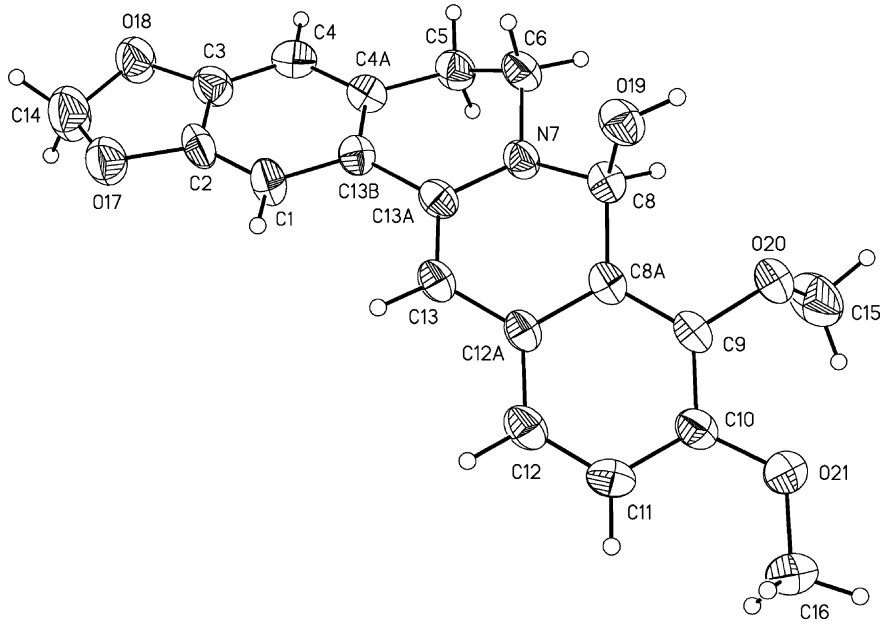

Fig. 3. A perspective view of 8-hydroxydihydroberberine (**2a**).

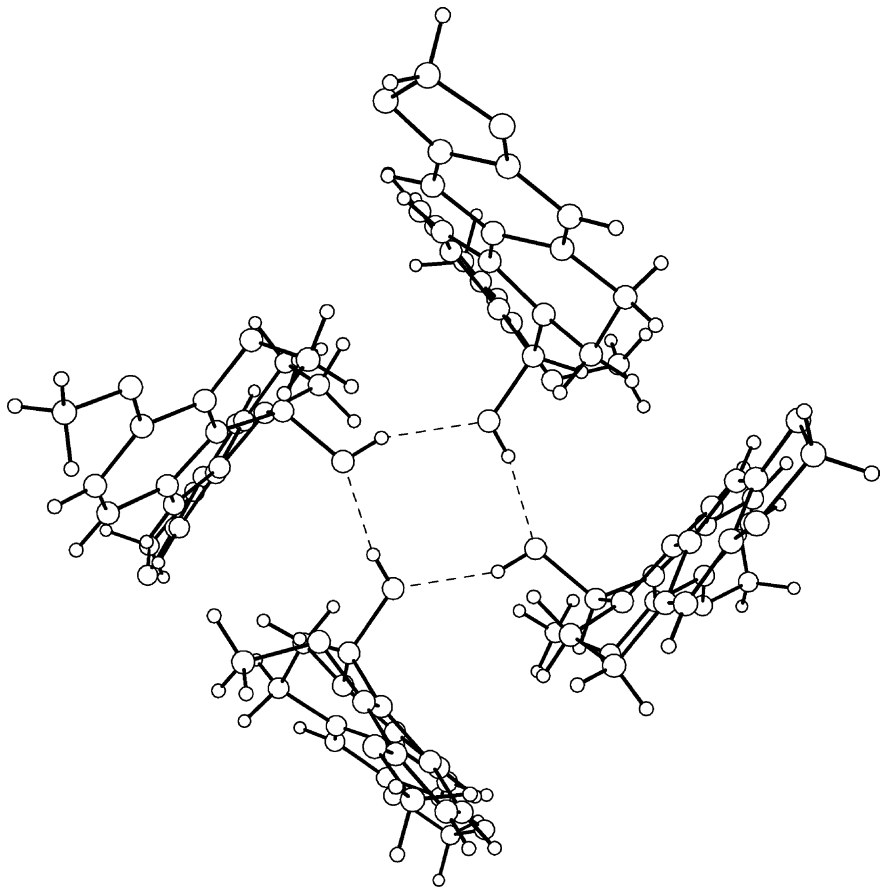

Fig. 4. Four H-bonded molecules of 8-hydroxydihydroberberine (**2a**).

Table 4
Hydrogen-bonding parameters (Å, deg) in the compound **2a**

D–H···A	D–H	H···A	D···A	D–H···A
O19–H19···O19′	0.84	1.93	2.756(4)	167.1
O19′–H19′···O19″	0.84	1.92	2.719(4)	158.2

D, donor atom; A, acceptor atom.

of sanguinarine [11] showed that the racemate is the preferable isomer. Therefore we expect that the dominant diastereomer could be **3a**. However, further measurements and/or calculations will be required to confirm this suggestion. To the best of our knowledge this is the first report of such a bimolecular derivative in protoberberine alkaloids. The ^{1}H and ^{13}C chemical shifts of compounds **3a** and **4a** are summarized in Tables 1 and 2. Because of their low concentrations only some H and C atoms were assigned. Fig. 2 shows a portion of GSQMBC spectrum [27] with C8/H8 crosspeaks of compounds **2a**, **3a**, and **4a**.

The ^{15}N NMR chemical shifts of 8-hydroxydihydroberberine (**2a**) and 8-oxoberberine (**5a**) δ 88.4 and 153.8 ppm, respectively, were published in our previous studies [28,29].

3.2. X-ray diffraction analysis of compounds *2a*, *5a*, and *5b*

Crystal data and refinement parameters including CCDC reference numbers of compounds **2a**, **5a**, and **5b** are listed in Table 3. In the crystal structure of **2a** there are two crystallographically unique molecules in the asymmetric unit. In the following, only one of those molecules is described and discussed. The second molecule is very similar, the only significant difference results from the conformational flexibility of the methoxy pendants, causing O20–C15 group (and its corresponding counterpart in the other molecule) to be rotated in the opposite directions. The *R*-values for **2a** are rather high, mainly due to a small crystal of lower quality, resulting into a lower diffracting power and relatively high number of unobserved reflections. Nevertheless, the residual electron density map does not show any significant features, thus we believe in model adequacy.

All bond lengths and angles in 8-hydroxydihydroberberine (**2a**) are within normal ranges. The C8–O19 bond distance is 1.440(5) Å. The sum of the three valence angles around nitrogen is 353.9°, which indicates that the hybridization of N gets closer to sp^2 rather than to sp^3. The partially hydrogenated heteroring B adopts a twisted half chair conformation while the ring C conformation resembles a shallow half chair. The atom C8 deviates from the plane calculated through C and D rings by 0.373(5) Å. The hemiaminoacetal hydroxyl group C8–OH is in axial position with respect to the ring C conformation (Fig. 3). The methoxy group attached to C9 atom is almost perpendicular to the plane D, while the other MeO group is nearly in-plane. Corresponding torsion angles are −117.7(5) [C8a–C9–O20–C15], 71.3(5) [C10–C9–O20–C15], 175.7(4) [C9–C10–O21–C16], and −3.6(6)° [C11–C10–O21–C16]. This is a common feature in tertiary berberine 8-adducts [5,8,12] as well as in quaternary

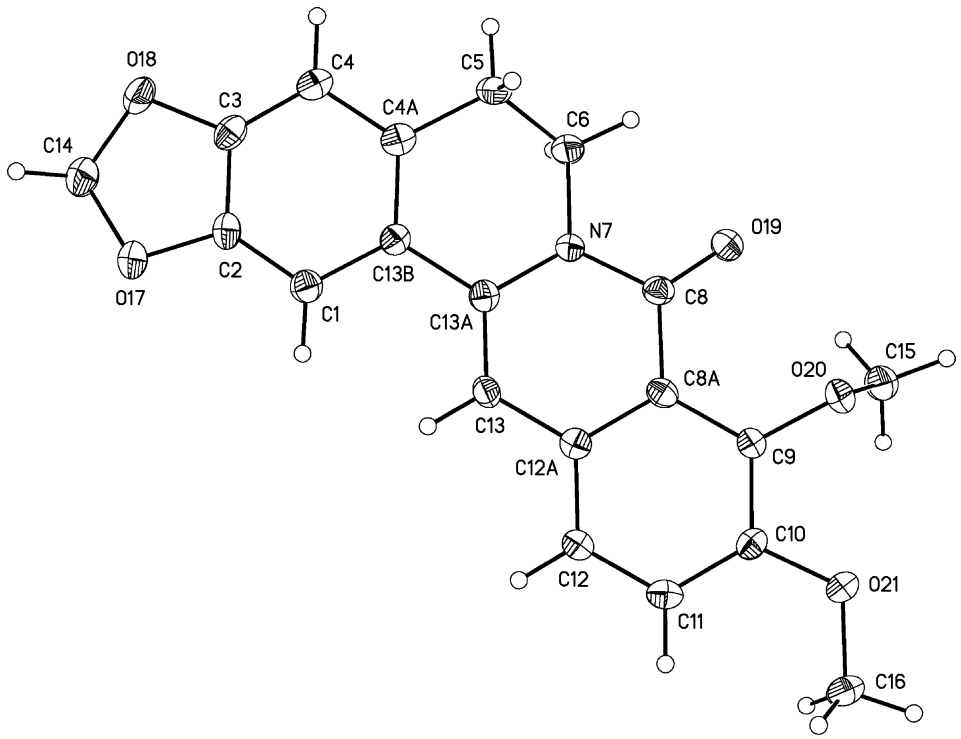

Fig. 5. A perspective view of oxoberberine (**5a**).

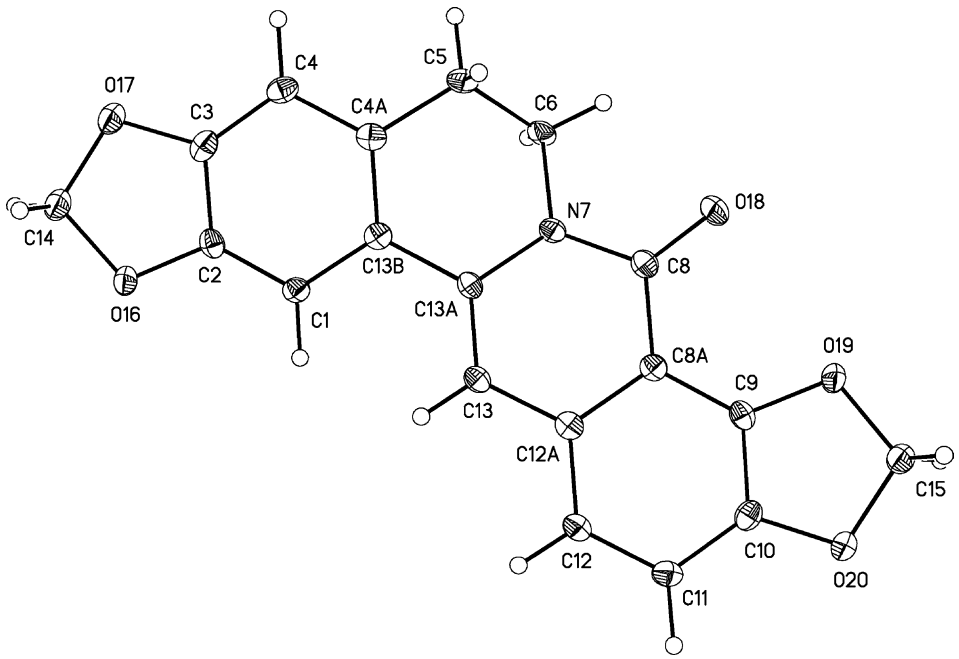

Fig. 6. A perspective view of oxocoptisine (**5b**).

berberine salts [5,30,31]. The dioxolane ring C2–C3–O18–C14–O17 is reasonably planar, with the mean deviation from a plane being 0.0511 Å. The angle between two aromatic rings A and D is 148.5(2)°.

The crystal structure of 8-hydroxydihydroberberine (**2a**) involves an interesting system of H-bonds. There are four H-bonds between hydroxyl groups linking four molecules of **2a** and giving rise to a central eight-membered heterocycle with alternating H and O atoms (Fig. 4). The geometric parameters of the hydrogen bonds are listed in Table 4. Hydroxyl groups are hidden inside four-molecular clusters and may be relatively protected against potential chemical attack in the solid phase, while in solution the compound **2a** rapidly reacts as it was shown during NMR measurements. The molecule of **2a** bears one stereogenic center, the carbon atom C8. Nevertheless, because of its synthetic origin, the compound was obtained as a crystalline racemate. To the best of our knowledge this is the first report on X-ray study of an isoquinoline-alkaloid pseudobase.

Contrary to 8-hydroxydihydroberberine (**2a**) the planarity of both 8-oxoberberine (**5a**, Fig. 5) and 8-oxocoptisine (**5b**, Fig. 6) is more pronounced, particularly in one of the two crystallographically independent molecules of oxoberberine. The planarity of all condensed ring systems is disturbed mainly by atoms C5 and C6 in ring B. All bond distances and angles are within normal ranges, the shortest one being in the C8=O oxo group: 1.232(2)/1.230(2) Å in **5a**, 1.235(1) Å in **5b**. The sum of bond angles around nitrogen N7 is 359.78/359.72 (**5a**) and 359.97° (**5b**), which corresponds to sp^2 hybridization. The two methoxy groups in **5a** are oriented in similar manner as in **2a**. The molecules of **5a** and **5b** are packed in columns along the *x* and *z* axis, respectively. There are no H-bonds, molecules are held together by van der Waals forces.

4. Conclusions

The free bases of quaternary protoberberine alkaloids berberine and coptisine are unstable 8-hydroxy adducts **2** (pseudobases). They are readily converted to salts **1** (by the action of acids), to 8-oxoderivatives **5** (in strongly alkaline environment) or to other adducts (in the presence of a reactive nucleophile). The structure of 8-hydroxydihydroberberine (**2a**) was supported by X-ray analysis. It was proven that, in non-polar solvent, bimolecular aminoacetal derivatives of berberine **3a** and **4a** can be formed.

Unlike quaternary salts of berberine (**1a**), 8-hydroxydihydroberberine (**2a**) is tested for its biological activities quite rarely. Recently the compound **2a** has been reported to be evaluated in cytotoxicity assays [32] and for antiprotozoal effects [33]. Unfortunately, the paper [32] does not state any characteristics of **2a**. There is some doubt about the identity of **2a** in the paper [33] because of preparation method (treatment with 10% HCl) and some differences in ^{1}H NMR chemical shifts with our data.

Acknowledgements

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