STRUCTURAL STUDIES OF CHELIRUBINE AND CHELILUTINE FREE BASES*

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The structures of chelirubine and chelilutine free bases have been examined by 2D NMR spectroscopy and mass spectrometry. Chelirubine chloride (**1a**) upon treatment with Na_2CO_3 yielded free base which possessed the constitution of bis(5,6-dihydrochelirubin-6-yl) ether (**2a**). The free base of chelilutine (**1b**) was determined to be bis(5,6-dihydrochelilutin-6-yl) ether (**2b**). The aqueous NH_3 treatment of chelilutine (**1b**) produced bis(5,6-dihydrochelilutin-6-yl)amine (**3b**). 6-Hydroxy-5,6-dihydrochelirubine (**4a**) and 6-hydroxy-5,6-dihydrochelilutine (**4b**) were detected only in $CDCl_3$ solution by NMR spectroscopy. In $CDCl_3$, the compound **2b** underwent hydrolysis to **4b** that was immediately followed by the reverse condensation to a diastereomer of **2b**. Pseudokinetics of this reaction was followed by NMR spectroscopy and quantum chemical calculations were carried out to support the suggested type of molecular symmetry alteration. The known alkaloid dihydrochelirubine (**5**) was isolated for the first time from *Sanguinaria canadensis*.

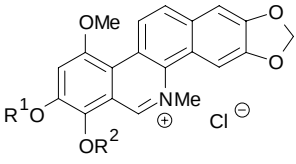
Key words: Chelirubine; Chelilutine; Quaternary benzo[*c*]phenanthridine alkaloids; Free bases; Pseudobases; Diastereoisomers; Dihydrochelirubine; NMR Spectroscopy; Mass spectrometry; Semiempirical calculations.

Chelirubine (**1a**) and chelilutine (**1b**) are two minor pentasubstituted quaternary benzo[*c*]phenanthridine alkaloids occurring in a number of species of the *Papaveraceae*

* Part IC in the series Alkaloids of the *Papaveraceae*; Part XCVIII: see ref.[1].

and *Fumariaceae* families[2]. They have been discovered by Slavík and Slavíková[3] in the root of *Chelidonium majus* L. during chromatographic separation of a sanguinarine-chelerythrine fraction. Structurally, chelirubine and chelilutine are methoxy derivatives of sanguinarine and chelerythrine[4], respectively. The unambiguous position of the methoxyl group at C-10 has been determined by total synthesis[5–8]. Quaternary chelirubine and chelilutine were also synthesized following biosynthetic pathways from protoberberine skeleton[9,10]. The identity of bocconine isolated later by Onda *et al.*[11,12] from *Macleaya cordata* (WILLD.) R. BR. (synonym: *Bocconia cordata* WILLD.) with chelirubine was confirmed by direct comparison[13].

Chelirubine and chelilutine display some biological activities, especially antimicrobial[11], antiparasitic[11], and antitumor[14] properties. Chelilutine inhibits α-DNA polymerase and reverse transcriptase[15], chelirubine and chelilutine inhibit acetylcholinesterase[16]. Chelirubine and chelilutine are easily reduced by NADH and NADPH coenzymes to form corresponding dihydro derivatives[17]. Their p*K* values, 7.70 and 8.50 for chelirubine and chelilutine, respectively, which reflect a succeptibility towards OH^- addition, were determined spectroscopically in aqueous environment[17]. Considering the p*K* value, chelirubine is the most reactive species of the six quaternary benzophenanthridine alkaloids studied[17]. Recently, chelirubine has been found to be a potent protein kinase C inhibitor[18].



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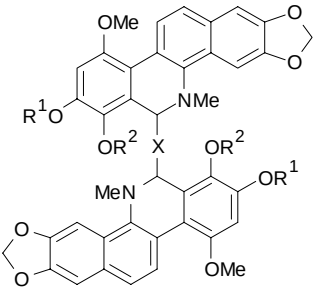
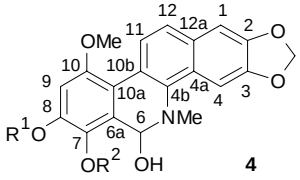
In formulae **1**-**4**

a, $R^1 + R^2 = CH_2$
b, $R^1 = R^2 = CH_3$



2, X = O
3, X = NH



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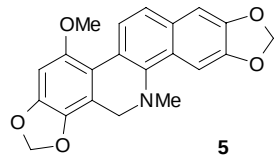

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Continuing our research on free bases* of quaternary benzophenanthridine alkaloids[19–23], we report here a detailed paper on free bases of chelirubine and chelilutine. Although the constitutions of the quaternary cations of chelirubine (**1a**) and chelilutine (**1b**) are known for more than twenty years the structures of their bases have not been elucidated to date.

EXPERIMENTAL

Melting points were determined on a Mettler FP 51 apparatus and are uncorrected. IR spectra were measured with an ATI Mattson GENESIS FTIR spectrometer in Na-dried Nujol unless indicated otherwise. ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer operating at frequencies of 500.13 (^{1}H), 125.76 (^{13}C), and 50.68 MHz (^{15}N), δ values are in ppm, coupling constants in Hz. Signals were referenced to TMS as an internal standard or to the signal of residual $CHCl_3$ (7.26 ppm) and $CDCl_3$ (77.00 ppm). 2D NMR experiments were carried out on a 5 mm triple-resonance probehead equipped with a gradient coil using pulse sequences described elsewhere (NOESY (ref.[24]), GE-HSQC (ref.[25]), and GSQMBC (ref.[26])). The ^{15}N chemical shifts were measured by the GSQMBC (ref.[26]) and GHMBC (refs[27–29]) experiments and referenced to the signal of liquid ammonia at 298 K used as an external standard. All NMR spectra were measured at 303 K. The AM1 calculations were performed on SGI Power Challange computer with R 10000 procesor using AMPAC 2.1 software.

Mass spectra were obtained with a Finnigan MAT TSQ 700 instrument in electron impact (70 eV) and desorption chemical ionization (DCI) mode using a 3 : 1 CH_4–N_2O mixture as the ionizing gas. Tandem mass spectrometry (MS/MS) was performed using Xe as collision gas at a pressure of 0.1 Pa with a collision offset voltage of 10 V. Chelirubine chloride (**1a**) (dark purple needles, m.p. 297–300 °C) was isolated from *Chelidonium majus* root[3] and *Sanguinaria canadensis* L. rhizomes[30] as reported previously. Chelilutine chloride (**1b**) (yellowish-orange needles, m.p. 191–192 °C) was obtained from alkaloidal fractions originated from *S. canadensis*[30]. The mixture of chelilutine and chelerythrine (approximately 1 : 1) was repeatedly separated by column chromatography on acid Al_2O_3 according to procedure described[30]. Ether used was entirely ethanol-free.

Bis(5,6-dihydrochelirubin-6-yl) Ether (**2a**)

A) Chelirubine chloride (**1a**, 30 mg) was dissolved in water and the solution was made alkaline with a saturated solution of Na_2CO_3. The precipitate was allowed to stand for 30 min at room temperature, then separated, washed with water and dried *in vacuo* (6 h, 95 °C). Yield: 25 mg of amorphous solid, m.p. 167–170 °C. IR spectrum (Nujol): 1 671, 1 575, 1 245, 1 201, 1 038, 930, 838 cm^{-1}. ^{1}H and ^{13}C NMR data ($CDCl_3$): see Table I. DCI MS, *m/z* (%): 741 (MH^+, 2), 740 (M^+, 2), 725 (9), 406 (2), 392 (10), 378 (20), 364 (100), 363 (93), 348 (31), 334 (7). MS/MS (740): 740 (100), 725 (8), 378 (36), 362 (53).

* The term *base* or *free base* has been universally adopted in the alkaloid chemistry for the product of basic properties which is formed upon alkalization of alkaloid salt and converts back to the original salt in acidic solutions. Free base formation is a reversible acidobasic reaction. The term itself is of no structural significance and, therefore, it has no relation to the organic nomenclature.

B) Chelirubine chloride (**1a**, 18 mg) was dissolved in water and the solution was made alkaline with a saturated solution of Na_2CO_3. The precipitate was extracted with ether. The organic layer was concentrated and allowed to stand to crystallize at room termperature. After 3 h colourless crystals were collected and dried *in vacuo* (2 h, 95 °C). Yield: 8 mg, m.p. 216–219 °C. Literature[30] gives m.p. 217–218 °C or 257–258 °C (capillary). ^{1}H and ^{13}C NMR data ($CDCl_3$): see Table I. DCI MS, *m/z* (%): 741 (MH^+, 7), 740 (M^+, 8), 725 (4), 709 (1), 408 (2), 392 (3), 380 (18), 378 (11), 362 (100), 348 (12). MS/MS (740): 362 (100).

Bis(5,6-dihydrochelilutin-6-yl) Ether (**2b**)

A) Chelilutine chloride (**1b**, 21 mg) was treated analogously as **1a** in the preparation of **2a** (method *A*). Yield: 18 mg of amorphous solid, m.p. 179–181 °C. IR spectrum (Nujol): 1 602, 1 240, 1 153, 1 039, 943 cm^{-1}. ^{1}H and ^{13}C NMR data ($CDCl_3$): see Table I. DCI MS, *m/z* (%): 773 (MH^+, 1), 772 (M^+, 3), 769 (2), 757 (3), 420 (3), 408 (9), 392 (36), 380 (82), 378 (75), 364 (100), 349 (5). MS/MS (772): 378 (100).

B) Chelilutine chloride (**1b**, 28 mg) was treated analogously as **1a** in the preparation of **2a** (method *B*). Yield: 12 mg, m.p. 228–229 °C. Literature[30] gives m.p. 229–230 °C (capillary). IR spectrum (Nujol): 1 604, 1 498, 1 329, 1 238, 1 207, 1 105, 1 026, 941 cm^{-1}. ^{1}H and ^{13}C NMR data ($CDCl_3$): see Table I. DCI MS, *m/z* (%): 773 (MH^+, 3), 772 (M^+, 4), 406 (4), 396 (11), 378 (100), 364 (18), 348 (3). MS/MS (772): 378 (100).

Bis(5,6-dihydrochelilutin-6-yl)amine (**3b**)

Chelilutine chloride (**1b**, 20 mg) was dissolved in water and the solution was made alkaline with concentrated aqueous ammonia. The precipitate formed was extracted with ether. The organic layer was concentrated and let stand to crystallize at room temperature. After 3 h colourless crystals were collected and dried *in vacuo* (2 h, 95 °C). Yield: 16 mg, m.p. 212–214 °C. IR spectrum (Nujol): 3 336, 1 599, 1 305, 1 153, 964 cm^{-1}. ^{1}H and ^{13}C NMR data ($CDCl_3$): see Table I. DCI MS, *m/z* (%): 772 (MH^+, 13), 771 (M^+, 18), 757 (1), 395 (6), 378 (100), 364 (17), 348 (3). MS/MS (771): 393 (20), 378 (100).

Isolation of Dihydrochelirubine (**5**)

Crude fractions of chelirubine (**1a**, 150 mg) from *S. canadensis*[30] were purified and crystallized from diluted HCl. During this operation colourless crystals of dihydrochelirubine (**5**, 12.4 mg) were separated in addition to purple crystals of **1a**. The crystals of **5** were washed with water and dried at room temperature, m.p. 189–190 °C. IR spectrum (KBr): 2 944, 2 888, 1 641, 1 617 cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.59 s, 3 H (OMe); 4.10 s, 2 H (H-6); 6.00 s, 2 H (7,8-OCH_2O); 6.03 s, 2 H (2,3-OCH_2O); 7.10 s, 1 H (H-1); 7.46 d, 1 H, J = 8.8 (H-12); 7.69 s, 1 H (H-4); 8.30 d, 1 H, J = 8.8 (H-11); ^{13}C NMR ($CDCl_3$): in entire accordance with ref.[33]. EI MS, *m/z* (%): 363 (M^+, 100), 362 (31), 348 ($M - Me^+$, 32), 332 (6), 320 (4), 181 (7). MS/MS (363): 363 (100), 362 (60), 348 (53).

RESULTS AND DISCUSSION

Free bases of the title alkaloids were prepared following two procedures: by simple precipitation from aqueous solution of natural quaternary alkaloid (method *A*) and by ether extraction of the reaction mixture and subsequent crystallization from non-polar solvent (method *B*). The conversion of brightly coloured quaternary salt **1** to colourless

free base is an immediate reaction. We used both methods to confirm or exclude whether the products of OH^- attack to the iminium bond are identical. The method *A* leads to white amorphous solid while the method *B* gives colourless crystalline compounds with considerably higher melting points. However, the method *A* provides better yield. Spectral data confirmed that the constitutions of free bases were identical regardless of the method used. Free bases of these alkaloids do not occur in acid environment of plant tissues and therefore they cannot be clasified as natural alkaloids.

The free base of chelirubine was subjected to desorption chemical ionisation mass spectrometry with solid sample introduction. There was reliably detected a pseudomolecular MH^+ ion at *m/z* 741 and a molecular ion at *m/z* 740 for bis(5,6-dihydrochelirubin-6-yl) ether (**2a**). The 725 and 709 peaks correspond to the loss of methyl $[M - 15]^+$ and methoxy $[M - 31]^+$ groups, respectively. The tandem mass spectrometry of the molecular ion showed a prominent peak at *m/z* 362 belonging to quaternary cation **1a**. In the IR spectrum, no presence of the O–H valence vibration band was observed. Similar conclusions apply to free base of chelilutine. DCI MS indicated the pseudomolecular and molecular species at *m/z* 773 and 772, respectively, for bis(5,6-dihydrochelilutin-6-yl) ether (**2b**). Thus, free bases of chelirubine and chelilutine are dimeric compounds **2a** and **2b**, respectively.

The ^1H and ^{13}C NMR data are summarized in Table I. In the ^1H NMR spectrum of **2a**, the singlet of H-6 (6.20 ppm) and the shielded signal of the 7,8-OCH_2O group (5.02, 5.54 ppm) are typical markers of the dimeric aminoacetal structure. Similarly, in **2b** the 7-OMe group was highly shielded (2.33 ppm) while the 8-OMe group displayed an expected chemical shift at δ 3.82. In order to make complete ^1H and ^{13}C assignments of all compounds a series of 1D and 2D NMR spectra was measured. Proton NMR signals were assigned by NOESY experiments. The chemical shifts of all protonated carbon atoms were determined using HSQC spectra. For an assignment of quaternary carbon atoms HMBC and GSQMBC pulse sequences optimized for the long-range coupling constants of 7 Hz were applied. In HSQC, HMBC, and GSQMBC pulse sequences the magnetic field gradient pulses were used for coherence pathways selection. The hydrogen atom signals of the OMe groups were assigned based on NOE spectra where their interactions with the skeleton protons were detected. The HMBC experiment indicated the interactions of the OMe hydrogen atoms with the C-7, C-8, and C-10 quaternary carbon atoms.

The action of aqueous ammonia on chelilutine chloride (**1b**) yielded bis(dihydrochelilutin-6-yl)amine (**3b**) similarly like in chelerythrine[19], sanguinarine[20], and sanguilutine[23]. In the IR spectrum, its N–H vibration band at 3 336 cm^{-1} appeared with extremely weak intensity. The ^1H NMR spectrum displayed analogical features in the shielding effect for the 7-OMe group (2.47 ppm) as in **2b**. The DCI mass spectrum of **3b** provided the pseudomolecular MH^+ (*m/z* 772) and molecular M^+ (*m/z* 771) species with relatively high intensities. The tandem mass spectrum of the molecular ion showed

splitting the MH^+ into two principal fragments: *m/z* 393 (cation **1b** + NH) and *m/z* 378 (cation **1b**). Reaction of chelirubine (**1a**) with NH_3 was not studied because of scarcity of material.

In the NMR spectra of compounds **2a** and **2b,** additional signals of related 6-hydroxy adducts (pseudobases) **4a** and **4b**, respectively, were recorded. These compounds are

TABLE I
^1H, ^{13}C, and ^{15}N NMR data of compounds **2**–**4** in $CDCl_3$

Atom	**4a**[a]		**2a**[a]		**4b**		**2b**[a]		**3b**	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	7.11	104.17	7.11	103.85	7.11	103.75	7.15	104.20	7.14	104.08
2	–	147.44	–	146.80	–	[b]	–	147.47	–	147.39
3	–	147.47	–	146.85	–	147.25	–	147.77	–	147.67
4	7.68	100.60	7.95	102.15	7.69	[b]	7.93	100.96	8.00	100.90
4a	–	127.14	–	127.31	–	[b]	–	126.77	–	127.61
4b	–	138.12	–	139.00	–	[b]	–	138.74	–	139.79
6	5.76[c]	79.01	6.20	78.97	5.97[d]	79.06	6.45	77.79	5.80	64.37
6a	–	115.58	–	114.60	–	128.74	–	128.28	–	130.84
7	–	139.15	–	139.05	–	140.67	–	140.36	–	139.97
8	–	147.88	–	147.04	–	153.77	–	153.44	–	153.32
9	6.69	95.84	6.44	95.57	6.66	98.56	6.42	97.98	6.41	97.33
10	–	152.57	–	152.16	–	152.35	–	152.21	–	152.07
10a	–	112.49	–	113.50	–	112.67	–	113.91	–	113.53
10b	–	122.03	–	122.71	–	123.32	–	122.51	–	122.51
11	8.45[e]	124.46	8.33[e]	124.75	8.44[f]	[b]	8.35[f]	124.65	8.28[f]	124.62
12	7.48[e]	123.18	7.39[e]	122.56	7.47[f]	122.95	7.42[f]	122.33	7.40[f]	122.23
12a	–	130.50	–	130.36	–	130.58	–	130.86	–	130.72
2,3-CH_2	6.05	101.84	6.07	100.74	6.04	[b]	6.10	100.69	6.09	100.87
7,8-CH_2	6.04	100.96	5.02	101.09	–	–	–	–	–	–
			5.54							
10-OMe	3.90	56.70	3.76	56.68	3.96	[b]	3.74	55.70	3.74	55.68
7-OMe	–	–	–	–	3.95	62.12	2.33	60.69	2.47	60.58
8-OMe	–	–	–	–	3.95	[b]	3.84	56.25	3.82	56.22
NMe	2.70	39.95	2.92	41.02	2.67	39.78	2.99	40.28	2.85	40.51
OH	2.10[c]	–	–	–	2.00[d]	–	–	–	–	–

[a] ^{15}N NMR shifts (δ, N-5), **4a**: 49.7; **2a**: 40.1; **2b**: 38.7. [b] Undetermined value. [c] Doublet, 1 H (J = 3.9 Hz). [d] Doublet, 1 H (J = 4.0 Hz). [e] Doublet, 1 H (J = 8.9 Hz). [f] Doublet, 1 H (J = 8.8 Hz).

products of aminoacetals **2** hydrolysis by residual water in the solvent. The signal of the semiaminal OH group is a doublet and the same splitting appeared for the H-6 hydrogen three atoms away. A clear interaction of OH and H-6 was also observed in the COSY spectrum. The ^{1}H and ^{13}C NMR data of compounds **4** are listed in Table I.

We have observed that the hydrolysis of bis(5,6-dihydrochelilutin-6-yl) ether (**2b**) in a $CDCl_3$ solution to 6-hydroxy-5,6-dihydrochelilutine (**4b**) is immediately followed by the reverse condensation to another product. The concentrations of reaction components were analysed directly from the ^{1}H NMR spectra as the integrals of the protons H-11, H-12, 7-OMe, and NMe. Figure 1 shows changes of the relative component concentrations measured by monitoring the corresponding NMe signals as a function of time. The observed dynamics indicates a slow inter-conversion process with an intermediate formation of pseudobase **4b** which after several hours leads to predominantly formed stereoisomer of **2b**. The most plausible explanation of the differences in ^{1}H NMR chemical shifts observed for both species is the existence of two diastereomers.

Compound **2b** possesses two stereogenic carbon atoms (C-6, C-6′) and therefore a racemate (6*S*,6′*S* + 6*R*,6′*R*) and a *meso*-form (6*S*,6′*R* ≡ 6*R*,6′*S*) are the two species to be considered. The *meso*-form of **2b** is preferentially formed during the preparation of free base in polar aqueous medium. Due to reaction conditions this process is fast and the formation of *meso*-**2b** is kinetically controlled. In $CDCl_3$ solution, the *meso*-**2b** is hydrolyzed by the residual water and 6-hydroxy-5,6-dihydrochelilutine (**4b**) is formed. This step is followed by the thermodynamically controlled condensation of two molecules of **4b** producing the final *racemate*-**2b** as the most stable species. Described type of molecular symmetry alteration is schematically shown in Scheme 1. In order to support hypothesis that *racemate*-**2b** is more stable than *meso*-**2b** quantum chemistry cal-

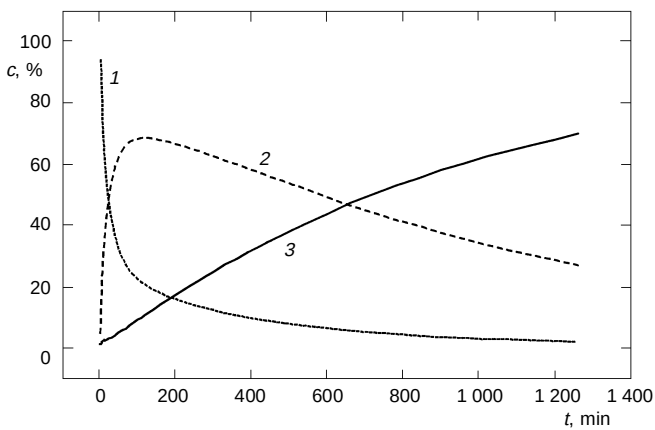
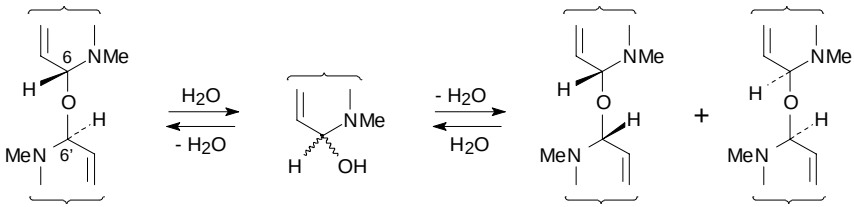


FIG. 1

Relative concentrations of components in the $CDCl_3$ solution of bis(dihydrochelilutin-6-yl) ether (**2b**) as a function of time for the NMe signals. *1* *meso*-**2b**, *2* **4b**, *3* *racemate*-**2b**

culations were performed. The calculations have revealed that *racemate*-**2b** is the lower energy diastereomer. The energy difference (ΔH^f) between these two diastereomers is 3.8 kcal/mol (15.9 kJ/mol). The AM1 optimized structures of both diastereomers are shown in Fig. 2. Analogical calculations in related alkaloids sanguinarine, chelerythrine, and chelirubine[22] showed that the racemate is a thermodynamically more stable stereoisomer. For the corresponding derivative of sanguilutine, the racemate was determined in crystal by X-ray analysis[23].

Thus, we suppose that the crystalline **2b** is a *meso*-form and its ^{1}H NMR data are in Table II. On the other hand, it seems that solid bis(dihydrochelirubin-6-yl) ether (**2a)** is a racemate. The $CDCl_3$ solution of **2a** contains the mixture of *racemate*-**2a**, pseudobase **4a**, and only traces of the second diastereomer (*meso*-**2a**) which was not assigned. The NMR data for *racemate*-**2a** and *racemate*-**2b** are summarized in Table I. Table II contains calculated differences in chemical shifts between *racemate*-**2b** and *meso*-**2b** as well as the comparison with the shifts of monomeric **4b**. From the data given it is evident that the greatest difference is observed for the shifts of the 7-OMe groups.



6*R*, 6'*S* 6*R*, 6'*R* 6*S*, 6'*S*

SCHEME 1

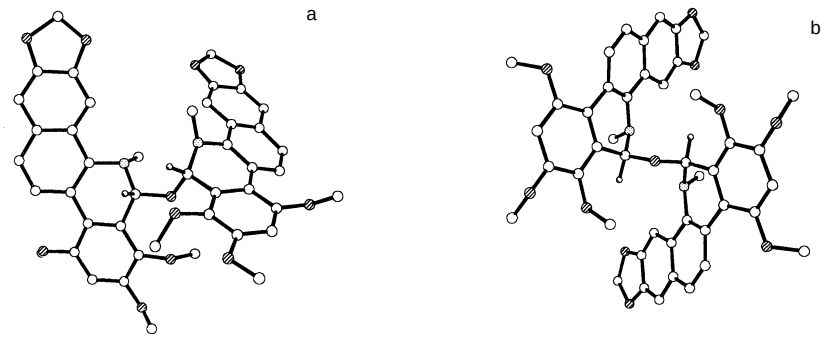


FIG. 2

An ORTEP plot of AM1 minimized structures of compounds 6*R*,6′*S*-**2b** (a) and 6*S*,6′*S*-**2b** (b) (hydrogen atoms with exception of H6 and H6′ are omitted for clarity)

Major differences are found also for the H-4, H-6, and NMe hydrogen atoms. The 7-OMe group in 6*S*,6′*S*-**2b** at δ 2.33 ppm is heavily shielded by the naphtalene moiety in the opposite half of the structure (Fig. 2) as compared to the neighbouring 8-OMe (δ 3.84 ppm). The AM1 calculations showed that the 7-OMe group is located directly above the centre of the 4a′–4b′–10b′–11′–12′–12a′ aromatic ring. No systematic study of conformational behaviour of the 7-OMe has been carried out. However, the distance between the 7-OMe carbon atom and the aromatic ring plane is approximately 4 Å. On the other hand, the 7-OMe group in *meso*-**2b** is located above the partially saturated ring (distance 3.9 Å) and is only mildly affected (δ 3.48 ppm) in comparison to the monomer **4b** (δ 3.95 ppm).

Our findings indicate that the formation chelirubine pseudobase **4a** is a consequence of the hydrolysis of **2a**. Konda *et al.*[31] reported the formation of 6-hydroxy-5,6-dihydrochelirubine (**4a**) from 10-nitro-6-methoxy-5,6-dihydrosanguinarine in a several-step transformation. They described the compound **4a** as colourless amorphous solid (m.p. 140–151 °C) using ^{1}H NMR data measured in $CDCl_3$ (a doublet at 5.55 ppm, J = 1.5 Hz, for the H-6 atom) and FAB MS (peak at *m/z* 362 for **1a**).

We have also obtained ^{1}H NMR spectra of **2a** and **2b** in C_6D_6. The spectrum of **2a** convincingly corresponded to that in $CDCl_3$; it showed signals of *racemate*-**2a** and **4a**, and traces of a *meso*-isomer. However, the composition and behaviour of the C_6D_6 solution of bis(dihydrochelilutin-6-yl) ether (**2b**) was quite different and more complicated comparing to $CDCl_3$ solution and will be object of further studies.

TABLE II
^{1}H NMR data of the *meso*-**2b** isomer and the differences in chemical shifts between compounds **2b** (racemic), *meso*-**2b**, and **4b** ($CDCl_3$)

Atom	*meso*-**2b**	$\delta_{meso\text{-}\mathbf{2b}} - \delta_{\mathbf{4b}}$	$\delta_{\mathbf{2b}} - \delta_{\mathbf{4b}}$	$\lvert \delta_{meso\text{-}\mathbf{2b}} - \delta_{\mathbf{2b}} \rvert$
1	7.17	0.06	0.04	0.02
4	7.06	–0.63	0.24	0.87
6	6.01	0.04	0.48	0.44
9	6.52	–0.14	–0.24	0.10
11	8.37	–0.07	–0.09	0.02
12	7.35	–0.12	–0.05	0.07
2,3-OCH_2O	6.04	0.00	0.06	0.06
7-OMe	3.48	–0.47	–1.62	1.15
8-OMe	3.88	–0.07	–0.11	0.04
10-OMe	3.83	–0.13	–0.22	0.09
NMe	2.47	–0.20	0.32	0.52

From the results presented, it is evident that not only the tetrasubstituted but also the pentasubstituted quaternary benzo[*c*]phenanthridine alkaloids of the sanguinarine type adopt dimeric aminoacetal structures in the form of free bases, as was supposed by Slavík[30] in 1960 (*cf.* also ref.[23]). The presence of pseudobases **4a** and **4b** was observed only in NMR spectra of their free bases **2a** and **2b**, respectively.

In addition, during the purification of the crude chelirubine fraction from the rhizomes of *Sanguinaria canadensis*, small amount of dihydrochelirubine (**5**) was obtained. Its structure was identified by comparing spectral and other physical data with a synthetically prepared sample[32]. This is the first report on dihydrochelirubine in *S. canadensis.* Untill now it was found only in *Chelidonium majus*[32]. Recently, Oechslin *et al.*[33] have isolated dihydrochelirubine from *Bocconia integrifolia* (HUMB. et BONPL.) DC. and provided its complete spectral data including ^{13}C NMR assignment.

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