Notes

Structure of Sanguinarine Base

Jiří Dostál,* Hana Bochořáková, Eva Táborská, and Jiří Slavík

Department of Biochemistry, Faculty of Medicine, Masaryk University, Komenského nám. 2, CZ-662 43 Brno, Czech Republic

Milan Potáček

Department of Organic Chemistry, Faculty of Science, Masaryk University, CZ-611 37 Brno, Czech Republic

Miloš Buděšínský

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, CZ-166 10 Praha, Czech Republic

Edmond de Hoffmann

Laboratory of Mass Spectroscopy, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium

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The structure of sanguinarine free base was examined. The base is either bis[6-(5,6-dihydrosanguinarinyl)] ether (**3**) or bis[6-(5,6-dihydrosanguinarinyl)]amine (**4**) depending on whether Na_2CO_3 or NH_3, respectively, is used as an alkalizing agent. Oxysanguinarine (**5**) was identified as a side product formed by disproportionation of the pseudobase intermediate **2a**.

The structure of the chelerythrine free base has been investigated in our recent work.[1] We report here that a related alkaloid, sanguinarine, also adopts a dimeric amino acetal structure. The current study, a followup to the previous paper,[1] focuses on a thorough elucidation of the sanguinarine base structure.

Sanguinarine (**1**) is a widely distributed member of the quaternary benzo[*c*]phenanthridine alkaloids occurring mainly in the Papaveraceae and Fumariaceae families.[2−4] This alkaloid exhibits considerable biological activity,[5−7] and it is commercially available. The name sanguinarine was introduced by Dana[8] in 1827 as the name for an alkaloid mixture from the rhizomes of *Sanguinaria canadensis* L. The pure alkaloid free of chelerythrine was obtained for the first time in 1924 by Gadamer and Stichel.[9]

The structure of the quaternary sanguinarine cation was resolved by Späth and Kuffner.[10] The sanguinarine base was generally assumed to be a 6-hydroxy adduct (pseudobase) **2a** but proper experimental data were lacking, although the amino acetal structure was proposed by Slavík and Slavíková[11] as early as 1960.

In order to prepare the sanguinarine free base, an aqueous solution of sanguinarine chloride (**1**) was made alkaline with Na_2CO_3. The precipitate formed was instantly extracted with ether, and the compound crystallized from the organic layer was identified as bis-[6-(5,6-dihydrosanguinarinyl)] ether (**3**). The composition of $C_{40}H_{28}N_2O_9$ was proven by elemental analysis. The calculated carbon content is 70.58% C for dimeric amino acetal **3**, and for monomeric aminohemiacetal **2a** it is 68.76% C. The found carbon content was 70.51%.

In the ^{1}H-NMR spectrum of **3** the signals of the H-6 atom (6.33 ppm) and the 7,8-OCH_2O group (δ 5.17 d, δ 5.66 d) were significant features differing from the NMR data of known sanguinarine derivatives.[2,12−14] The unusual highfield position of the 7,8-OCH_2O doublets is probably caused by an anisotropic effect of the second unit of the dimer. The aromatic H-atoms were assigned in a ^{1}H−^{1}H-COSY NMR experiment. The chemical shifts of the carbon atoms were assigned using the APT spectrum and by comparison with the spectrum of the analogous chelerythrine base.[1] In the ^{1}H- and ^{13}C-NMR spectra, the signals of **3** were accompanied by signals of another component. The complete assignment revealed that this was the pseudobase **2b** (Table 1). Compound **2b** is a regular product of the amino acetal **3** hydrolysis due to HDO being present in the $CDCl_3$ used. This is the first NMR assignment of this pseudobase (**2b**).

The NMR data of the prevailing diastereomer of **3** are listed in Table 1. There were some low-intensity signals in the ^{1}H-NMR spectrum of **3** that might be ascribed to another diastereomer of **3**. Compound **3** possesses two chiral atoms (C-6, C-6′), and during its formation a mixture of three stereoisomers may be expected. The two enantiomers 6*S*,6′*S* + 6*R*,6′*R* form a racemate having the same NMR spectra. One more isomer represents the meso-form (6*S*,6′*R* ≡ 6*R*,6′*S*). The racemate and the meso-form are in diastereomeric relation, and therefore, they are expected to display slightly different chemical shifts in the NMR spectrum. The amount of the minor isomer is estimated as up to 10%. The existence of stereoisomers in chelerythrine base has

* To whom correspondence should be addressed. Phone: 42-5-42126509. FAX: 42-5-42126200. E-mail: jrdostal@med.muni.cz.

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Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **2b**, **3** and **4**[a]

	compound					
	2b		**3**		**4**	
position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1,1′	7.12 s	104.53	7.13 s	104.26	7.12 s	104.14
2,2′		147.32[b]		147.07[c]		147.10[d]
3,3′		147.57[b]		147.19[c]		147.34[d]
4,4′	7.66 s	100.73	7.96 s	102.24	7.99 s	102.16
4a,4′a		127.07		127.25		128.12
4b,4′b		137.94		138.91		139.60
6,6′	5.83 s	78.85	6.33 s	79.24	5.75 s	65.59
6a,6′a		113.77		112.87		115.06
7,7′		145.26		145.06		144.54
8,8′		148.22[b]		147.00[c]		147.04[d]
9,9′	6.95 d (8.2)	108.91	6.71 d (8.0)	108.33	6.68 d (8.5)	107.65
10,10′	7.43 d (8.2)	116.51	7.23 d (8.0)	115.69	7.20 d (8.5)	115.74
10a,10′a		122.28		122.98		123.26
10b,10′b		125.20		125.97		125.75
11,11′	7.77 d (8.6)	120.09	7.65 d (8.5)	120.42	7.64 d (8.5)	120.31
12,12′	7.50 d (8.6)	124.07	7.43 d (8.5)	123.45	7.44 d (8.5)	123.55
12a,12′a		131.03		130.90		130.96
OCH_2O	6.07 d (1.6)	101.83	6.08 d (1.3)	100.91	6.09 br s	100.88
	6.12 d (1.6)	100.91	6.11 d (1.3)	101.11	6.12 br s	101.09
	6.05 d (1.2)		5.17 d (1.3)		5.31 br s	
	6.05 d (1.2)		5.66 d (1.3)		5.69 br s	
NMe,N′Me	2.74 s	40.60	2.98 s	41.89	2.86 s	42.03

[a] Recorded at 500 MHz (^{1}H) and 125.7 MHz (^{13}C) in $CDCl_3$; shifts in ppm. Coupling constants (in Hz) are given in parentheses. [b–d] Values with the same superscript may be interchanged.

not to date been observed,[1] though it has never been discounted.

Moreover, the further singlets at δ 3.90, 2.62, and 4.20 in the ^{1}H-NMR spectrum of **3** indicated[2,12] the presence of oxysanguinarine (**5**) and dihydrosanguinarine (**2c**) as the products of pseudobase **2b** disproportionation. Their OCH_2O and aromatic H signals were mostly overlapped and, therefore, could not be assigned.

The sanguinarine base precipitated by Na_2CO_3 without Et_2O extraction was a compound whose ^{1}H-NMR spectrum was identical with that of **3**. Repeated crystallization of compound **3** from EtOH yielded 6-ethoxy-5,6-dihydrosanguinarine (**2d**).

CIMS of compound **3** showed a quasimolecular ion at *m/z* 681 (M + 1). The base peak at *m/z* 332 corresponded to the quaternary cation **1**. The fragment at 665 (M − 15) was a result of demethylation of **3**. The fragment at *m/z* 318 arose from the demethylation of sanguinarine (**1** − CH_3 + H^+). The ion with *m/z* 348 represented **1** with an oxygen in position 6. The tandem mass spectrum of the 665 ion displayed peaks at *m/z* 332, 318, 304, and 274, which is a typical fragmentation pattern of 6-substituted derivatives of dihydrosanguinarine.[2,12,13]

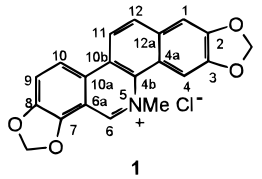
In the presence of acids, **3** easily converts to the bright red quaternary cation **1**. The Et_2O mother liquor afforded a small amount of a compound that had a conspicuously high mp of above 350 °C and did not change in the presence of acids. The IR and MS data of this product were identical with the spectra of reference oxysanguinarine (**5**). EIMS of **5** showed molecular species at *m/z* 347 (radical cation) and *m/z* 346 (M − H^+). In the mother liquor, dihydrosanguinarine (**2c**) was also detected by HPLC. The derivatives **2c** and **5** were formed by disproportionation of the primary hydroxy adduct **2a** as it occurs in simple 5-methylphenanthridinium cation.[15]

It is known that only the 8,9-substituted quaternary benzo[*c*]phenanthridine alkaloids, nitidine and avicine, yield oxo and dihydro derivatives under alkaline conditions.[16,17] This is the first report on the spontaneous oxysanguinarine formation in alkaline media. To verify this discovery, we performed the following experiment. To a small amount of sanguinarine chloride (**1**) in H_2O were added a few drops of aqueous Na_2CO_3, and the reaction mixture was studied using HPLC. The immediate analysis with the strongly acid mobile phase showed only a peak of sanguinarine. The analysis after 6 h showed the peaks of oxysanguinarine (**5**) and dihydrosanguinarine (**2c**) in addition to sanguinarine peak. The experiment thus provides indirect evidence for the presence of the pseudobase **2a** in the reaction system. The adduct **2a** both condenses to **3** and, to a very small extent, disproportionates to **5** and **2c**. Analogous conversion was not observed in the case of the chelerythrine pseudobase.[1] The formation of side products **2c** and **5** implies that sanguinarine is a more reactive system than chelerythrine, a statement that has been cited on a number of occasions.[4]

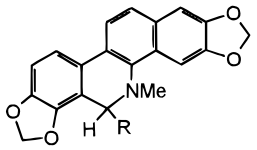
Oxysanguinarine and dihydrosanguinarine were isolated from a number of species in the Papaveraceae family.[2,18,19] Dihydrosanguinarine has been recently, for the first time, isolated in a small amount from the rhizomes of *S. canadensis*.[20]

Treatment of sanguinarine chloride (**1**) with an excess aqueous NH_3 in a similar way as for **3** yielded a nitrogen analogue bis[6-(5,6-dihydrosanguinarinyl)]amine (**4**). Elemental analysis confirmed a higher content of nitrogen, which is in agreement with the three N atoms present. The IR spectrum of **4** displayed a diagnostically significant valence vibration of the NH bond at 3372 cm^{-1}.

The ^{1}H- and ^{13}C-NMR data of the major isomer of **4** are presented in Table 1. As in **3**, the moisture in $CDCl_3$ caused the hydrolysis of **4** with the formation of pseudobase **2b** and aminal **2e**. There were some more low-intensity signals in the ^{1}H-NMR spectrum of **4**, which might be attributed to the minor diastereomer of **4**. Because of the complexity of the spectrum we could not resolve the NMR data of **2e** and the minor isomer of **4**.



1



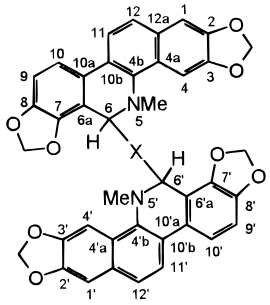
2a: R = OH

2b: R = OD

2c: R = H

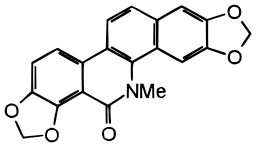
2d: R = OEt

2e: R = NHD



3: X = O

4: X = NH



5

The following singlets at δ 2.49 (NMe), 2.63 (NMe), 5.16 (H-6), and 5.23 (H-6) probably belonged to these minor components.

CIMS of the compound **4** showed a quasimolecular ion at m/z 680 (M + 1) and a molecular peak at m/z 679. The tandem MS of the molecular ion displayed fragments at m/z 633 (loss of a methylenedioxy group, M − 46) and at m/z 347 (**1** + NH).

Experimental Section

General Experimental Procedures. Melting points were determined on a Mettler FP 51 apparatus and are uncorrected. Diffuse reflectance infrared FT (DRIFT) spectra were measured with a Nicolet Impact 400 FT-IR spectrophotometer in a micro cuvette. ^{1}H-, ^{13}C-, and 2D-NMR spectra were recorded on Tesla BS 587A (80/20 MHz) and Varian UNITY-500 (500/125.7 MHz) spectrometers in $CDCl_3$, δ values are reported in ppm downfield relative to TMS as an internal standard, J in Hz. For mass spectra and elemental analysis determinations see Dostál *et al.*[1]

HPLC Conditions. HPLC system consisted of an Ecom high pressure pump LCP 3001, an ECOM UV detector LCD 2040 (detection at 280 nm), an Apex integrator, a Rheodyne 7125 sample injector with a 50-μL sample loop (Cotati) and a Separon SGX C18 (5 μm, 3 × 150 mm, Tessek) column. The mobile phase consisted of two solvents (*A*,*B*). Solvent A contained triethylamine in redistilled H_2O (0.1 mol/L), buffered to pH 2.5 with H_3PO_4; B was MeCN. Isocratic elution was performed with 60% B in A, flow rate 0.5 mL/min.

Chemicals. Sanguinarine chloride (**1**) was isolated from the rhizomes of *S. canadensis* L. as described previously.[11] The purity of **1** was checked by TLC and HPLC. Oxysanguinarine (**5**) standard was available from isolation.[18] Dihydrosanguinarine (**2c**) standard was prepared[21] by the reduction of sanguinarine chloride (**1**) with $NaBH_4$. The Et_2O used was entirely free of EtOH. Triethylamine and MeCN were of HPLC grade (Sigma).

HPLC Analysis. Sanguinarine chloride (**1**, 2.5 mg) was dissolved in H_2O (10 mL). The solution was made alkaline with a few drops of Na_2CO_3 solution (pH 10). The reaction mixture was left in a tightly sealed test tube at room temperature. After 6 h, in addition to the main peak of **1**, the peaks of oxysanguinarine (**5**) and dihydrosanguinarine (**2c**) were recorded. During 24 h no other changes appeared on the chromatogram. The compounds **5** and **2c** were identified by comparing their retention times with those of standards and by coinjection of standards to the sample analyzed. Retention times (min): sanguinarine (**1**) 5.41; oxysanguinarine (**5**) 12.85; dihydrosanguinarine (**2c**) 26.52.

6-Ethoxy-5,6-dihydrosanguinarine (2d). The compound was obtained after repeated crystallization of **3** from the mixture of $CHCl_3$−EtOH (1:10): mp 210−211 °C; IR (DRIFT) ν max 2977, 2896, 1641, 1605, 1499, 1466, 1444, 1362, 1321, 1254, 1189, 1040, 983, 946, 859 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 80 MHz) δ 1.10 (3H, t, J = 7.1 Hz, C-Me), 2.76 (3H, s, NMe), 3.56−4.04 (2H, m, CH_2), 5.48 (1H, s, H-6), 6.04−6.12 (4H, m, 2 × OCH_2O), 6.91 (1H, d, J = 8.3 Hz, H-9), 7.12 (1H, s, H-1), 7.40 (1H, d, J = 8.3 Hz, H-10), 7.46 (1H, d, J = 8.6 Hz, H-12), 7.66 (1H, s, H-4), 7.77 (1H, d, J = 8.6 Hz, H-11); after D_2O addition the signals of **2b**, 2.74 (3H, s, NMe), 5.83 (1H, s, H-6), and the signals of CH_3CH_2OD were recorded; EIMS m/z [M]$^+$ 377 (4), 332 (21), 166 (4); MS/MS (377, positive mode) 332 (100); MS/MS (332, positive mode) 332 (100), 304 (46), 274 (65), 246 (33), 218 (13).

Bis[6-(5,6-dihydrosanguinarinyl)] Ether (3). Sanguinarine chloride (**1**, 164 mg) was dissolved in H_2O, and the solution was made alkaline with a saturated solution of Na_2CO_3. The precipitate formed was extracted with ether. The organic layer was concentrated and allowed to stand at room temperature. After 6 h colorless crystals were collected and dried *in vacuo* (**3**, 58 mg). From the mother liquor 60 mg of an unidentified crystalline product (mp 155−157 °C) was obtained. Compound **3**: mp 258−260 °C; IR (DRIFT) ν max 2958, 2888, 1648, 1610, 1471, 1368, 1307, 1264, 1252, 1240, 1193, 1156, 1124, 1102, 1059, 1042, 994, 938, 900 cm^{-1}; ^{1}H- and ^{13}C-NMR data of the major isomer ($CDCl_3$), see Table 1; ^{1}H-NMR data of the minor isomer ($CDCl_3$, 500 MHz) δ 2.59 (3H, s, NMe), 5.68, 5.90 (2H, 2 × d, J = 1.0 Hz, 7,8-OCH_2O), 6.26 (1H, s, H-6), aromatic H-atoms: 6.87 (1H, d, J = 8.0 Hz), 7.08 (1H, s), 7.37 (1H, d, J = 8.0 Hz), 7.73 (1H, d, J = 8.0 Hz), the remaining signals were overlapped; CIMS m/z [M + 1]$^+$ 681 (0.24), [M]$^+$ 680 (0.20), 665 (0.42), 663 (0.30), 651 (0.06), 649 (0.09), 362 (7), 348 (11), 334 (56), 332 (100), 318 (33); MS/MS (665, positive mode) 665 (100), 346 (48), 332 (92), 318 (20), 304 (8), 274 (3). Anal. Calcd for $C_{40}H_{28}N_2O_9$: C, 70.58; H, 4.15; N, 4.12. Found: C, 70.51; H, 4.27; N, 4.16.

Treatment of 1 with Aqueous Na_2CO_3. Sanguinarine chloride (**1**, 31 mg) was dissolved in H_2O, 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 filtered off, washed with water, and dried *in vacuo* (24 mg); mp 241−244 °C; ^{1}H-NMR spectrum ($CDCl_3$) was identical with that of **3**.

Bis[6-(5,6-dihydrosanguinarinyl)]amine (4). Sanguinarine chloride (**1**, 270 mg) was dissolved in H_2O, and the solution was made alkaline with concentrated

aqueous NH_3. The precipitate was extracted with ether. The organic layer was concentrated and allowed to stand at room temperature. After 2 days, colorless crystals of **4** were collected and dried *in vacuo* (150 mg). From the mother liquor 46 mg of an unidentified crystalline product with mp 157−158 °C was obtained. Compound **4**: mp 264−266 °C; IR (DRIFT) ν max 3372 (w, NH), 2964, 2892, 1648, 1609, 1500, 1467, 1415, 1361, 1289, 1247, 1230, 1186, 1155, 1124, 1100, 1091, 1035, 938, 900 cm^{-1}; ^1H- and ^{13}C-NMR data ($CDCl_3$), see Table 1; CIMS *m*/*z* $[M + 1]^+$ 680 (10), $[M]^+$ 679 (10), 664 (0.3), 332 (8); MS/MS (679, positive mode) $[M - 46]^+$ 633 (8), 347 (39), 332 (100), 304 (3). Anal. Calcd for $C_{40}H_{29}N_3O_8$: C, 70.69; H, 4.30; N, 6.18. Found: C, 70.55; H, 4.23; N, 6.15.

Oxysanguinarine (5). The mother liquor after crystallization of **3** afforded oxysanguinarine, which was recrystallized from MeOH−$CHCl_3$ (3.2 mg): mp 354−356 °C; IR (DRIFT) ν max 2920, 2850, 1652 (s, C═O), 1646, 1600, 1506, 1478, 1320, 1290, 1261, 1208, 1129, 1047, 940, 872, 812, 781, 669, 600 cm^{-1}; EIMS *m*/*z* $[M]^{\bullet+}$ 347 (64), 346 (100), 318 (15), 288 (9), 274 (6), 232 (4), 203 (4), 188 (6), 159 (13), 137 (4); MS/MS (347, positive mode metastable) 347 (100), 318 (3); MS/MS (318, positive mode) 318 (100), 316 (18), 290 (46), 288 (32), 272 (22), 260 (45), 244 (50), 232 (37), 216 (50), 204 (16), 189 (7); MS/MS (288, positive mode) 288 (100), 260 (92), 232 (72), 204 (55), 202 (17), 176 (9).

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