# MEDICINAL PLANTS

## DISPROPORTIONATION OF THE ALKALOID SANGUINARINE ON SILICA GEL

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Sanguinarine (I) and chelerythrine (II) are components of the antimicrobial preparation sanguritrine [1, 2]. In studying the conditions for chromatographic separation of these alkaloids on silica gel using chloroform or ethyl acetate as eluents, we have observed, besides the products isolated previously [3, 4], small amounts of two additional substances not detected in the initial sum of alkaloids: fraction F represented by a low-polarity colorless band producing violet fluorescence upon exposure to UV light (362 nm), and fraction G – a colorless band producing blue fluorescence under UV irradiation and having a chromatographic mobility close to that of sanguinarine. Therefore, the two substances are artifacts arising in the course of the chromatographic procedure. In choosing the optimum separation conditions and selecting eluates, we have established that the behavior of these artifacts depend on the method of alkaloid application onto the sorbent, the time of contact with the sorbent, the nature of eluent, etc.

When the above conditions were modified and a mixture of sanguinarine (I) and chelerythrine (II) hydrosulfates upon treatment with an aqueous ammonia solution was applied onto a silica gel and, after a contact time of 24 h, eluted with toluene or toluene – methanol mixtures (from 0 to 10% MeOH), the leading band containing substance F (with violet fluorescence) markedly increased, while the initial fraction of I (visible orange band) was virtually absent. The spot of this substance upon TLC on Silufol UV-254 plates exposed to the UV light changes the fluorescence color from violet to orange and becomes visible in the daylight. Repeated TLC run of the converted substance in the perpendicular direction in parallel with a reference sample of sanguinarine bisulfate revealed their equal chromatographic mobility and the same fluorescence color. This susceptibility to photochemical oxidation and the change in the visible and fluorescence color are characteristic of dihydrosanguinarine (III) [5]. When compound III was obtained in a preparative amount and analyzed by $^1$H NMR, the sample spectrum coincided with the known spectrum of 5,6-dihydrosanguinarine (see Table 1)

The following fractions comprise a mixture of the trace amounts of sanguinarine (I) with dihydrosanguinarine (III), chelerythrine (II), and traces of dihydrochelerythrine. The latter is confirmed by the fact that, upon exposure to light, the chromatogram reveals an additional band (below sanguinarine and above chelerythrine spots) having the same visible and fluorescence colors as chelerythrine.

The formation of 5,6-dihydrosanguinarine (III) was explained in the preliminary communication [4] as resulting from a redox transformation (disproportionation) of sanguinarine on the surface of silica gel (Fig. 1). In complete agreement with this hypothesis, we have isolated from the more polar fractions the substance G in the form of thin needles

**TABLE 1.** Characteristics of the $^1$H NMR Spectra of Sanguinarine (I) [5], 5,6-Dihydrosanguinarine (III), and 6-Oxo-5,6-dihydrosanguinarine (IV)

| Compound | $^1$H NMR chemical shift δ, ppm (J, Hz) | | |
|---|---|---|---|
| | I | III | IV |
| $H^1$ | 7.78 s | 7.11 s | 7.26 s |
| $H^4$ | 8.28 s | 7.67 s | 7.54 s |
| $H^6$ | 10.15 s | 4.20 s | – |
| $H^9$ | 8.13 d (8.0) | 6.86 d (8.1) | 7.38 d (8.6) |
| $H^{10}$ | 8.65 d (8.0) | 7.30 d (8.1) | 7.88 d (8.6) |
| $H^{11}$ | 9.80 d (9.0) | 7.69 d (8.6) | 8.04 d (8.8) |
| $H^{12}$ | 8.31 d (9.0) | 7.48 d (8.6) | 7.67 d (8.8) |
| N–$CH_3$ | 4.92 s | 2.62 s | 4.02 s |
| $OCH_2O$ | 6.35 s | 6.04 s | 6.20 s |
| | 6.61 s | 6.05 s | 6.14 s |

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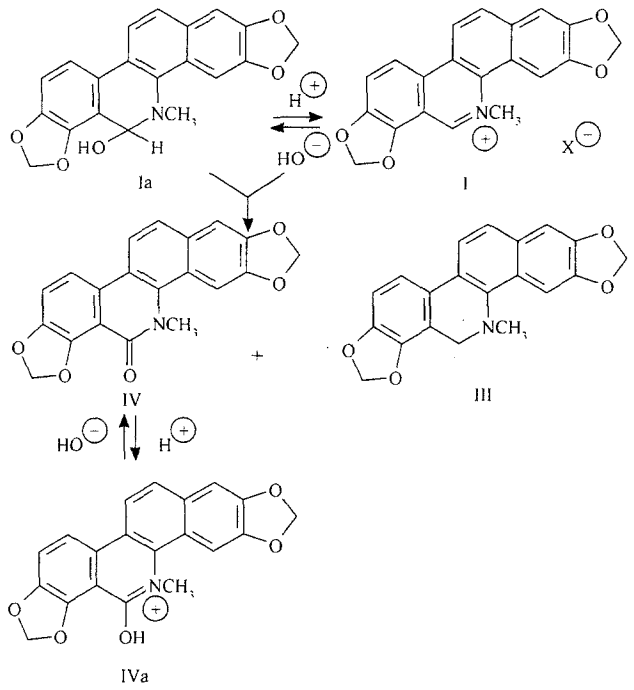

**Fig. 1.** Empirical scheme of redox transformations of sanguinarine (I).

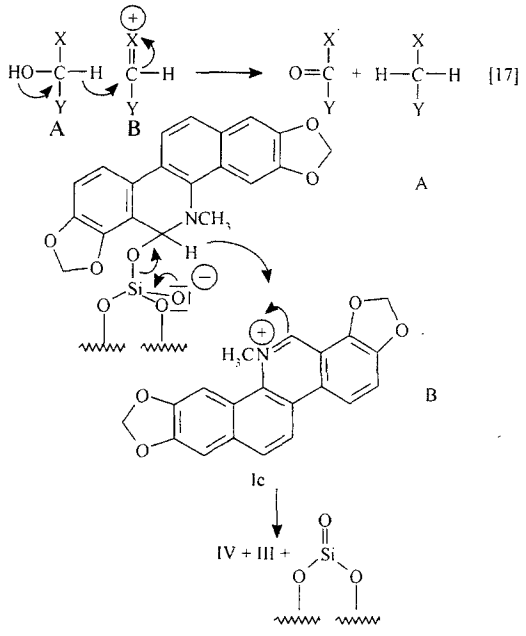

**Fig. 2.** Schematic diagram illustrating the mechanism of disproportionation of sanguinarine (I).

having m.p. = 360 – 361°C (from chloroform). The $^1$H NMR spectrum of this compound measured in a $CDCl_3$ – $CF_3COOH$ (2 : 1) solution corresponded to the spectrum of 6-oxo-5,6-dihydrosanguinarine (IV), with characteristic absence of a signal from the H6 proton.

The pattern of chemical shifts of the proton signals in this spectrum apparently corresponds to a protonated form of the alkaloid (IVa), in which the signal from protons of the N–$CH_3$ group (4.02 ppm) is shifted by 0.9 ppm toward stronger fields as compared to an analogous signal in the spectrum of sanguinarine (as a result of the electron-donor effect of the OH group), while the other signals are shifted by 0.60 – 0.75 ppm.

A partial disproportionation of sanguinarine was previously observed by Dostal et al. [6] upon the reaction of sanguinarine chloride with sodium carbonate. It was established that, while avicine and nitidine (8,9-isomers of compounds I and II) are readily disproportionated in an $NH_4OH$ medium [7], sanguinarine forms under the same conditions only a dimeric base of bis(5,6-dihydro-6-sanguinarinyl)amine [6]. At the same time, no disproportionation of I was observed upon the sanguinarine treatment with an aqueous or aqueous – alcohol NaOH solution in the range of pH 3.5 – 11.5 over a period of up to 120 days [8]. Previously, compound IV was obtained by oxidizing I with $K_3Fe(CN)_6$ in an alkaline medium [9] and by other methods, while compound III was synthesized by reducing I with $NaBH_4$ [10, 11] or with a zinc powder in an acid medium [12]. Both these compounds (III and IV) represent the natural products isolated from the plants of *Chelidonium majus* [13, 14] and *Sanguinaria canadensis L.* [15], the cell cultures of *Papaver somniferum* and *Maclea cordata* [16], and from other sources.

Habermehl and Schunck [17] described disproportionation of a pseudobase of the alkaloid hydrastinine on $Al_2O_3$ in a benzene solution and proposed a mechanism for this reaction. This includes interaction of the pseudobase (aminoalcohol) A with a quaternary form (immonium ion) B occurring in the state of dynamic equilibrium. During the disproportionation reaction, ion A serves as the donor of hydride ions and the quaternary salt B – as the acceptor of these ions (Fig. 2).

Thus, a necessary condition for the disproportionation reaction to proceed is the presence of a sufficient amount of both A and B ions, their ratio being dependent on the pH value of the medium. The equilibrium between pseudobase and quaternary salt for benzo[c]phenanthridine alkaloids in a strongly alkaline medium shifts almost completely toward the pseudobase formation, and that in a strongly acidic medium – toward the quaternary salt. Therefore, no disproportionation takes place under these conditions. However, according to [8], the coexistence of anions A and cations B in the medium is not a sufficient condition for disproportionation. For this reason, no data were reported on the formation of products of the sanguinarine transformation in the course of separation of alkaloids I and II on columns filed with $Al_2O_3$ [18] and silicic acid [19].

Under our experimental conditions, the excess ammonium upon the treatment of sanguinarine and chelerythrine salts is removed and the surface of silica gel (having a weak acid properties) provides conditions for the coexistence of both forms – donor (A) and acceptor (B) – of hydride ions.

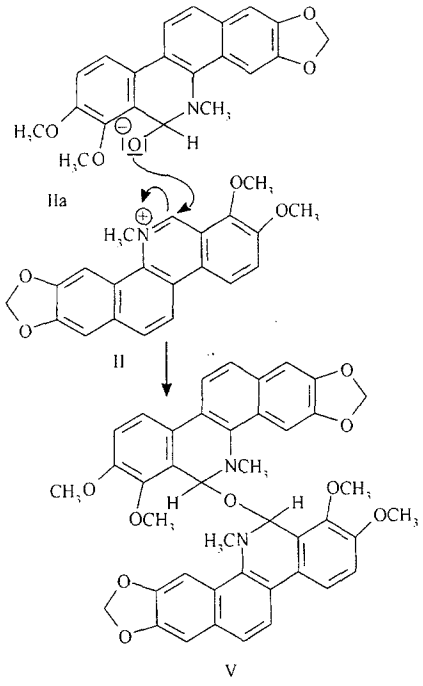


Fig. 3. Empirical scheme of formation of bis-(5,6-dihydro-6-chelerythryl)oxide (V).

This is confirmed by the data of TLC in a silica gel layer for various times of contact between alkaloid and sorbent. Another important factor is the nature of eluent. The neutral toluene medium features the transformation of alkaloid I, while the polar chloroform and acidified ethyl acetate media suppress the reaction. As a result, the conversion is insignificant, probably because of solvation of the pseudobase of I with chloroform or a shift of the equilibrium toward the immonium form of I in ethyl acetate. Disproportionation of some other benzo[c]phenanthridine alkaloids (avicine and nitidine) was reported in low-polarity solvents (benzene) [7].

Activation of the disproportionation process on silica gel can be explained as follows. On the sorbent surface, the sanguinarine pseudobase (Ia) forms a salt with silicic acid (Ic, X = $-SiO_3^-$). In an aprotic organic solvent (toluene), this salt exhibits an anionotropic rearrangement (similar to that described for sanguinarine acetate [20]) with the formation of a silicic ether of the sanguinarine pseudobase. As a result, a cyclic siloxane transition complex [A + B] (Ic) is formed on the silica gel surface. This complex is characterized by easy detachment of the hydride ion in position $C^6$ (Fig. 2) resulting in the disproportionation.

We have observed the formation of similar transformation products, albeit to an insignificant extent, in the case of chelerythrine (II). This alkaloid was isolated from the last fractions by chromatography on silica gel and had the form of a dimer with the formula V (Fig. 3). The $^1H$ NMR spectrum of compound V fully coincided with that reported in [21] (δ, ppm): 7.15 (s, $H^1$, $H^{1'}$), 7.95 (s, $H^4$, $H^{4'}$), 6.61 (s, $H^6$, $H^{6'}$), 6.85 (d, J 8.7 Hz, $H^9$, $H^{9'}$), 7.46 (d, J 8.7 Hz, $H^{10}$, $H^{10'}$), 7.70 (d, J 8.5 Hz, $H^{11}$, $H^{11'}$), 7.46 (d, J 8.5 Hz, $H^{12}$, $H^{12'}$), 6.15 (bs, $OCH_2O$), 3.07 (s, $NCH_3$ and $N'CH_3$), 3.74 and 2.44 (s, $OCH_3$).

Thus, the pseudobases of sanguinarine (Ia) and chelerythrine (IIa) are subject to various transformation under the same conditions on the silica gel surface: the former compound predominantly exhibits disproportionation and the latter – dimerization. The susceptibility to $C^6O$- or $C^6$-deprotonation depends on the electron density distribution in the aromatic system of molecules of alkaloids I and II and, hence, on the bacisity of nitrogen atoms in these molecules. In particular, the more basic chelerythrine apparently occurs in a quaternary form on the sorbent surface under the separation conditions. This form is not subject to any significant anionotropic rearrangement. The corresponding pseudobase, which is susceptible to proton detachment from the hydroxy group, has the form of an oxide anion and interacts with the quaternary form of II to form a dimer with the formula V (Fig. 3).

A quaternary salt of the weaker base sanguinarine (I) converts on the silica gel surface in toluene into a $C^6$–O-silyl derivative susceptible to detachment of the hydride ion from the $C^6$–H position. This results in disproportionation of the initial compound. The proposed mechanism of this reaction is depicted in Fig. 2. In the absence of silica gel, the formation of a cyclic transition state involving I and Ia is probably sterically hindered by substituents in positions 7 and 8, preventing the molecules from approaching the "active centers" (attack of the electrophilic center $C^6$ in I must proceed in a plane perpendicular to the plane of the benzo[c]phenanthridine system). However, there are no steric hindrances for the interaction of fragments A and B in the transition state Ic, leading to the corresponding dimeric bases: bis-dihydrosanguinaryl oxide (VI) and amine (VII).

To the present, extensive experimental knowledge has been gained concerning the course of heterogeneous processes on the surface of hydrated silicon dioxide (silica gel), but the mechanism of catalysis of these reactions is not always clear. Basyuk [22] noted that there is analogy in the behavior of some substances on the surfaces of $Al_2O_3$ and $SiO_2$ and suggested that the transition complexes may be stabilized by functional groups tightly bound to the active surface centers.

The observed reaction of sanguinarine disproportionation formally resembles the Meerwein – Ponndorf – Verley reaction (oxidation with carbonyl compounds) proceeding in the presence of aluminum alcoholates [23, 24].

In our opinion, the above-described disproportionation of sanguinarine chloride treated with $Na_2CO_3$ [6] can be explained by the same mechanism involving a cyclic transition state with a carbonate fragment linking two alkaloid molecules (I and Ia) by ionic and covalent bonds. The increased susceptibility of nitidine and avicine to disproportionation is probably related to easier access of the electrophilic center for the nucleophilic attack in their molecules having no substituents in position 7. This assumption is apparently confirmed

by easy disproportionation of 5-methylphenanthridine iodide in silica gel in an aprotic solvent [25].

## EXPERIMENTAL PART

The $^1$H NMR spectra were measured on a Gemini 200 (Varian, USA) spectrometer operating at a working frequency of 200 MHz, using TMS as the internal standard and $CDCl_3$ or $CDCl_3 - CF_3COOH$ as solvents. The purity of compounds and the fraction compositions were checked by TLC on Silufol UV-254 plates eluted in a diethyl ether – petroleum ether (70 – 100°C) – methanol (35 : 15 : 3) system (a 25% aqueous ammonia solution was added into the starting sample spot). The substances were identified by the color of visible (daylight) and UV-induced (362 nm) fluorescence and by the data of $^1$H NMR spectroscopy.

**Isolation of 5,6-dihydrosanguinarine (III).** A mixture of sanguinarine (45%) and chelerythrine (53.2%) bisulfates (1.5 g) was dissolved in a mixture of 2.5 ml chloroform and 6 ml of 25% aqueous ammonia. The solution was mixed with 7.0 g of silica gel (40 / 100 μm grade), triturated, and dried for 24 h at room temperature. The column (50 × 3 cm) was filled by portions with a mixture of same silica gel (150 g) with toluene (300 ml) and allowed to stand for densification for 24 h. Then the above sanguinarine – chelerythrine – silica gel mixture was applied onto the top of the chromatographic column. The elution is performed in the following sequence: 300 ml toluene, 300 ml of a 3% methanol solution in toluene, 300 ml of a 7% methanol solution in toluene, and 1250 ml of a 10% methanol solution in toluene. The sequential 75 – 85 ml fractions were collected. The first fractions 1 – 4 (~350 ml) represent pure eluent and fractions 5 – 8 (340 ml) contain 5,6-dihydrosanguinarine III (by TLC data: compound III possesses a higher chromatographic mobility than sanguinarine and exhibits violet fluorescence under UV irradiation, the color changing to orange). Upon solvent evaporation, the latter fractions yield an amorphous mass containing compound III identified by the $^1$H NMR spectrum (Table 1). Fraction 9 (90 ml) contains a mixture of compound III with traces of sanguinarine (I).

**Isolation of 6,6′-bis-(5,6-dihydrochelerythryl)oxide (V)** Fractions 10 – 12 (300 ml) were partly evaporated to obtain 0.2 g of a colorless crystalline substance with m.p. = 254 – 257°C. According to the $^1$H NMR data, this product is bis-(5,6-dihydro-6-chelerythryl)oxide (V).

**Isolation of 6-oxo-5,6-dihydrosanguinarine (IV).** Fractions 13 – 17 (750 ml) contain a mixture of trace amounts of chelerythrine (by the data of TLC in the system indicated above – the product of dimer decomposition) and its dihydro derivative. Fractions 18 – 23 were used to isolate 0.085 g of crystalline 6-oxo-5,6-dihydrosanguinarine (IV) with m.p. = 360 – 361°C (from chloroform). Compound IV was identified by $^1$H NMR spectroscopy.

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