# QUATERNARY BENZO[*c*]PHENANTHRIDINE ALKALOIDS

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This review deals with the fully aromatic quaternary benzo[*c*]phenanthridine alkaloids in several aspects. Firstly nomenclature principles for numbering fused heterocycles are given. Then the richest botanical sources of quaternary benzo[*c*]phenanthridines are mentioned as well as isolation, separation and determination methods are presented. The emphasis of this survey is focused on chemical reactivity. The main pharmacological effects of quaternary benzo[*c*]phenanthridine alkaloids are discussed.



## 1. INTRODUCTION

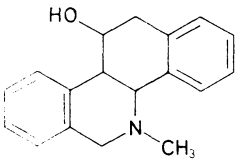
Alkaloids form structurally a very heterogeneous group of secondary metabolites with limited and specific distribution among living organisms[1]. The alkaloid com-

position of a plant species belongs to the typical and biogenetically conditioned features of higher plants. These biochemical features can serve for the systematic characterization of a taxon in the same way as the morphological characters.

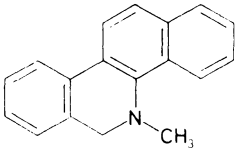
The benzo[*c*]phenanthridine alkaloids, commonly called as benzophenanthridine alkaloids, belong to a large group of isoquinoline alkaloids biosynthesized from phenylalanine[2,3].

Benzo[*c*]phenanthridines represent a widely spread type of alkaloids in the *Papaveraceae*, *Fumariaceae* and *Rutaceae* families. At present the benzo[*c*]phenanthridine alkaloids are studied in many world laboratories. The greatest attention is devoted to their biological activity and pharmacological properties. The results of isolations have a great importance for biosynthesis studies and chemotaxonomy.

A number of reviews discussing these alkaloids have been published, however, the presented review deals with the fully aromatic quaternary benzo[*c*]phenanthridine alkaloids (QBPAs) in more detail than the other papers emphasizing their chemical reactivity.




*A*

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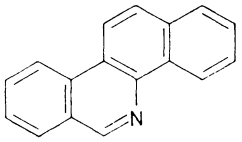
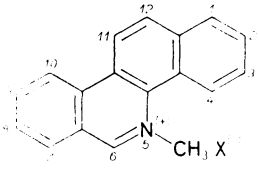
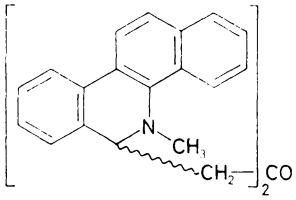


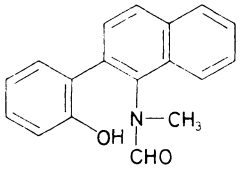

*C*




*D*




*E*




*F*

Recently[4] Šimánek has published a review on all constitutional types of benzophenanthridine alkaloids including among others their stereochemistry, synthesis and biosynthesis. In 1984 a detailed survey of spectral data and botanical sources of 88 benzophenanthridine alkaloids was issued[5]. Ninomiya and Naito[6] have reported a comprehensive review on syntheses of the benzo[*c*]phenanthridine skeleton. Preininger[7] has summarized the distribution of benzo[*c*]phenanthridines in the *Papaveraceae* and *Fumariaceae* families. There are also several reviews on pharmacological properties of benzo[*c*]phenanthridine alkaloids[8–11]. In recent years

interactions of QBPAs with biopolymers[12,13] (e.g. enzymes, nucleic acids) as well as their biosynthesis and tissue culture production have been intensively studied[14,15].

Six constitutional types of benzo[*c*]phenanthridine alkaloids so far have been known: hexahydrobenzo[*c*]phenanthridine alkaloids A, dihydrobenzo[*c*]phenanthridine alkaloids B, N-demethylbenzo[*c*]phenanthridine alkaloids C, quaternary benzo[*c*]phenanthridine alkaloids D, dimeric dihydrobenzo[*c*]phenanthridine alkaloids E, secobenzo[*c*]phenanthridine alkaloids F.

Benzo[*c*]phenanthridines described in the literature can be of three origines: (i) natural alkaloids isolated from plants as a product of metabolism, (ii) artifacts formed during an isolation procedure, (iii) synthetically prepared benzo[*c*]phenanthridines.

At present the number of quaternary benzo[*c*]phenanthridine alkaloids (QBPAs) described in the literature is about 14, of synthetic quaternary benzo[*c*]phenanthridines about 35 and of other benzo[*c*]phenanthridine alkaloids about 70.

Compounds *1–14* (see Table I) are alkaloids isolated from plants and compounds *15–49* (see Table II) are quaternary benzo[*c*]phenanthridine salts prepared by synthesis.

## 2. NOMENCLATURE

The trivial names of QBPAs used in the literature have been mostly derived from the Latin names of the plant species and the colour of their salts. They are conventionally assigned to the quaternary (iminium) form naturally occurring in plant tissues. The systematic names of alkaloids are not often used in most of the papers due to their length. For example sanguinarine (*1*) is the name of the 2,3,7,8-bis-(methylendioxy)-5-methylbenzo[*c*]phenanthridinium cation regardless of the accompanying anion. Full information is included in the name e.g. sanguinarine chloride. Besides, the combined nomenclature is common in case of some derivatives: 5,6-dihydrosanguinarine, 6-oxosanguinarine, etc. There are several ways of numbering the benzo[*c*]phenanthridine skeleton in the literature. The principles for numbering fused heterocycles are given by "IUPAC Nomenclature of Organic Chemistry", 1971, the rule A-22 for numbering fused polycyclic hydrocarbons. See also the review[16] "The Nomenclature of Heterocycles".

The rules can be expressed in four principles: the polycyclic system is oriented so that: (i) the maximum number of rings is in the horizontal axis *x* and (ii) in the upper right quadrant demarcated by axes *x* and *y*. (iii) If more orientations fulfil these requirements, that one is chosen which has the minimum number of rings in the lower left quadrant. (iv) The numbering (in a clockwise direction) is started on the tertiary carbon atom in the most counterclockwise position of the uppermost ring, or if there are more posibilities, of the uppermost ring farthest to the right. The quaternary carbon atoms are not numbered.

This situation is illustrated for phenanthridine (9-azaphenanthrene) in Fig. 1 and for benzo[*c*]phenanthridine (5-azachrysene) in Fig. 2.

The parent hydrocarbon of phenanthridine – phenanthrene is an exception to rule A-22.

TABLE I

Structures of benzo[*c*]phenanthridine alkaloids

| Alkaloid | Position of substituents[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 3 | 7 | 8 | 9 | 10 | 12 |
| Sanguinarine (*1*) | $OCH_2O$ | | $OCH_2O$ | | H | H | H |
| Chelerythrine (*2*) | $OCH_2O$ | | OMe | OMe | H | H | H |
| Chelirubine (*3*) | $OCH_2O$ | | $OCH_2O$ | | H | OMe | H |
| Chelilutine (*4*) | $OCH_2O$ | | OMe | OMe | H | OMe | H |
| Macarpine (*5*) | $OCH_2O$ | | $OCH_2O$ | | H | OMe | OMe |
| Avicine (*6*) | $OCH_2O$ | | H | $OCH_2O$ | | H | H |
| Nitidine (*7*) | $OCH_2O$ | | H | OMe | OMe | H | H |
| Sanguirubine (*8*) | OMe | OMe | $OCH_2O$ | | H | OMe | H |
| Sanguilutine (*9*) | OMe | OMe | OMe | OMe | H | OMe | H |
| Fagaronine (*10*) | OH | OMe | H | OMe | OMe | H | H |
| Fagaridine (*11*) | $OCH_2O$ | | OH | OMe | H | H | H |
| Punctatine (*12*)[b] | OR | OR | OR | OR | H | H | H |
| 7,8-Demethylene-sanguinarine (*13*) | $OCH_2O$ | | OH | OH | H | H | H |
| 7,8-Demethylene--7,8-dehydro-sanguinarine (*14*) | $OCH_2O$ | | =O[c] | =O[c] | H | H | H |

Me methyl. [a] For numbering see structure *D*; [b] 3 × Me and 1 × H; [c] part of *o*-quinoid system.

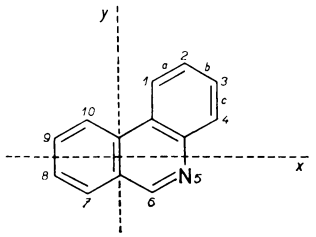



FIG. 1

The numbering of phenanthridine

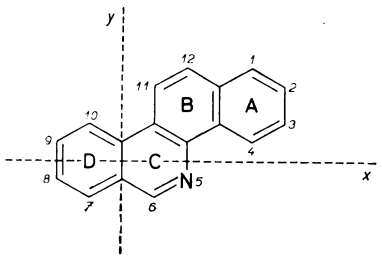



FIG. 2

The numbering of benzo[*c*]phenanthridine

TABLE II

Structures of benzo[*c*]phenanthridine derivatives

| Compound | Position of substituents[a] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Ref. |
| *15* | $OCH_2O$ | | OMe | H | OMe | OMe | H | H | H | H | 17 |
| *16* | $OCH_2O$ | | H | H | OH | OH | H | H | H | H | 18 |
| *17* | OMe | OMe | H | Me | H | OMe | OMe | H | OAc | OAc | 19 |
| *18* | $OCH_2O$ | | H | H | H | OH | OMe | H | H | H | 20 |
| *19* | $OCH_2O$ | | H | H | H | OMe | OH | H | H | H | 20 |
| *20* | $OCH_2O$ | | H | $NH_2$ | $OCH_2O$ | | H | H | H | H | 21 |
| *21* | $OCH_2O$ | | H | Cl | $OCH_2O$ | | H | H | H | H | 21 |
| *22* | OMe | OMe | H | H | H | $OCH_2O$ | | H | H | H | 22 |
| *23* | OMe | OMe | H | H | H | OMe | OMe | H | H | H | 22 |
| *24* | OH | OH | H | H | H | OH | OH | H | H | H | 22 |
| *25* | OAc | OAc | H | H | H | OAc | OAc | H | H | H | 22 |
| *26* | H | H | H | H | H | OMe | OMe | H | H | H | 23 |
| *27* | OMe | OMe | H | H | H | H | H | H | H | H | 23 |
| *28* | OMe | OMe | H | H | H | H | OMe | H | H | H | 23 |
| *29* | OMe | OMe | H | H | H | OMe | H | H | H | H | 23 |
| *30* | H | OBz | H | H | H | OMe | OMe | H | H | H | 23 |
| *31* | H | OH | H | H | H | OMe | OMe | H | H | H | 23 |
| *32* | OMe | OMe | H | H | H | OMe | OEt | H | H | H | 23 |
| *33* | OMe | OMe | H | H | H | OEt | OMe | H | H | H | 23 |
| *34* | OEt | OEt | H | H | H | OMe | OMe | H | H | H | 23 |
| *35* | OPr-i | OMe | H | H | H | OMe | OMe | H | H | H | 23 |
| *36* | OMe | OPr-i | H | H | H | OMe | OMe | H | H | H | 23 |
| *37* | OMe | OMe | H | H | H | OPr-i | OMe | H | H | H | 23 |
| *38* | OMe | OMe | H | H | H | OMe | OPr-i | H | H | H | 23 |
| *39* | OMe | OH | H | H | H | OMe | OMe | H | H | H | 23 |
| *40* | OMe | OMe | H | H | H | OH | OMe | H | H | H | 23 |
| *41* | OMe | OMe | H | H | H | OMe | OH | H | H | H | 23 |
| *42* | $OCH_2O$ | | H | Me | H | OMe | OMe | H | H | H | 24 |
| *43* | $OCH_2O$ | | H | H | OMe | OMe | OMe | H | H | H | 24 |
| *44* | $OCH_2O$ | | H | H | H | OMe | OMe | OMe | H | H | 24 |
| *45* | $OCH_2O$ | | H | H | OMe | H | $OCH_2O$ | | H | H | 24 |
| *46* | OBz | OMe | H | H | H | OMe | OMe | H | H | H | 25 |
| *47* | $OCH_2O$ | | H | H | H | OPr-i | OMe | H | H | H | 20 |
| *48* | $OCH_2O$ | | H | H | H | OMe | OPr-i | H | H | H | 20 |
| *49* | OMe | OMe | H | H | H | OMe | OMe | OMe | H | H | 26 |

Me methyl, Et ethyl, Ac acetyl, Bz benzyl, Pr-i isopropyl. [a] For numbering see structure *D*.

## 3. BOTANICAL SOURCES

Benzo[*c*]phenanthridine alkaloids are found in many plants of *Papaveraceae*, *Fumariaceae*, *Rutaceae* and other families. For a detailed survey on the alkaloid composition of individual plant species see refs[4,7,27]. From the quantitative standpoint the three richest sources of sanguinarine and chelerythrine types of alkaloids can be mentioned: *Sanguinaria canadensis* L., *Dicranostigma lactucoides* HOOK. F. et THOMS., *Chelidonium majus* L. (*Papaveraceae*).

### *Sanguinaria canadensis* L.

*Sanguinaria canadensis* L. is the only species of the genus *Sanguinaria* L. It is a perennial herb commonly spread in the eastern parts of Nothern America from Florida to Quebec and in the west to the Mississipi River. It particularly occurs in cool and shaded woods of the Appalachian Mountains.

In American literature the plant is usually called bloodroot. Other names are red root, puccon root and tetterwort.

*Sanguinaria* was one of the first species investigated for their alkaloid content due to its conspicuous appearance. The fresh plant sheds an intensively red latex (the colouring caused by QBPAs). Sanguinaria extract has been used for medicinal purposes by American Indians since long ago. For a review on the early literature see ref.[28].

This plant species is the richest source of QBPAs at all. Rhizomes contain 3–7% of total alkaloids depending on the environmental conditions and the time of collection[29]. The QBPAs form about 90% of total alkaloids. Slavík[28] isolated 6 QBPAs from the commercial sanguinaria drug in the total yield 2·65%: sanguinarine (*1*), chelerythrine (*2*), chelirubine (*3*), chelilutine (*4*), sanguirubine (*8*), and sanguilutine (*9*). The quantitative proportions of individual alkaloids are given in Table III.

TABLE III

The ratio of quaternary benzo[*c*]phenanthridine alkaloids isolated from *Sanguinaria canadensis* L. (in %)

| Alkaloid | Ref.[28] | Ref.[29] |
|---|---|---|
| Sanguinarine (*1*) | 40·3 | 50 |
| Chelerythrine (*2*) | 36·9 | 25 |
| Sanguilutine (*9*) | 10·0 | 15 |
| Chelilutine (*4*) | 8·6 | 5 |
| Chelirubine (*3*) | 2·7 | 4 |
| Sanguirubine (*8*) | 1·3 | 1 |

On the other hand the leaves of *S. canadensis* contain about 0·06% of QBPAs only[30].

*Chelidonium majus* L.

*Chelidonium majus* L., greater celandine, is a perennial herb commonly widespread in Central Europe. Since long ago it has been employed as a medicinal plant with a wide range of using. The fresh plant sheds a deep orange latex. In addition to other substances it contains a considerable number of alkaloids[27]. The total content of alkaloids depends on various external conditions especially on the vegetation period[31]. The highest alkaloid content in the root is found at the end of the vegetation period (September–October).

In the root total of alkaloids varies around 2·5%. The main alkaloid (+)-chelidonine forms 40–70% of all alkaloids and it is easy to isolate as a poorly soluble hydrochloride[32]. Chelidonine can be converted to sanguinarine (*1*) via oxidation of O-acetylchelidonine with mercury(II) acetate[33,34].

The amount of the QBPAs in the root ranges from about 0·4% to 0·8%[31,32,35]. Sanguinarine (*1*), chelerythrine (*2*), chelirubine (*3*) and chelilutine (*4*) have been found in this species. The alkaloid content in the overground part is about ten times lower than that in the root (0·2%). The QBPAs form 0·05% only[36].

*Dicranostigma lactucoides* HOOK. F. et THOMS.

The genus *Dicranostigma* HOOK. F. et THOMS. comprises three species: *Dicranostigma lactucoides* HOOK. F. et THOMS., *Dicranostigma leptopodum* (MAXIM.) FEDDE, *Dicranostigma franchetianum* (PRAIN) FEDDE. All of them are herbs occuring in the eastern Himalayas and central China. Relatively few authors have been engaged in the study of these species in contrast to *S. canadensis* and *Ch. majus*. Besides there

TABLE IV

The content of quaternary benzo[*c*]phenanthridine alkaloids in some plant species

| Plant species | Underground part | Overground part | Reference |
|---|---|---|---|
| *Sanguinaria canadensis* L. | 2·60–2·74% | 0·06% | 28, 30, 39 |
| *Chelidonium majus* L. | 0·4–0·8% | 0·05% | 31, 32, 35, 36 |
| *Dicranostigma lactucoides* HOOK. f. et THOMS. | 1·4–1·8% | 0·003% | 37, 38 |

are inaccuracies in the systematic determination of *D. franchetianum* species in early literature (cf. commentary in ref.[37]).

*D. lactucoides* is a biennal herb which is not difficult to cultivate in the Central European geoclimatic conditions. The root is the main source of alkaloids; it contains more than 2% of total alkaloids, 1·4–1·8% of QBPAs (ref.[38]). Accordingly, the root of *D. lactucoides* represents the second richest source of QBPAs (Table IV). The overground part contains about 0·4% of total alkaloids, mainly isocorydine and a little QBPAs (0·003%). In other species of the genus *Dicranostigma* the content of QBPAs is considerably lower.

## 4. ISOLATION AND ANALYTICAL METHODS

### 4.1. Extraction and Isolation

The probably first mention of these alkaloids is found in the literature in 1828 when Dana[40] isolated a sum of alkaloids from *Sanguinaria canadensis* L. and called it sanguinarine. In last century another very spread benzo[*c*]phenanthridine alkaloid chelerythrine was discovered in *Chelidonium majus* L. The chronological survey of the isolation and structural establishment of fourteen QBPAs is given in Table V.

Methanol appears to be the most suitable solvent to extract an alkaloid fraction from the dried plant material. The extraction procedure can be carried out either in cold countercurrently (percolation) or with hot solvent in a Soxhlet extractor. After evaporation of the solvent a crude extract results which is usually a very complex mixture of many organic and inorganic substances. This extract must be put in diluted acid. After alkalization the alkaloid fraction is extracted with non-polar solvents (ether, chloroform).

The QBPAs are easy to extract from the alkaline aqueous solution with diethyl ether where they pass in form of pseudobases together with other tertiary alkaloids. There are three main ways of how to isolate QBPAs from the alkaloid mixture. The choice of the method depends on the nature of the plant species and further on qualitative and quantitative characters (known or supposed) of alkaloid content.

(i) The simplest case is to separate the QBPAs from an alkaloid fraction as less soluble quaternary salts of inorganic acids from the corresponding acid solution. Thus, 10% sulfuric acid was used to isolate the mixture of sanguinarine (*1*) and chelerythrine (*2*) hydrogensulfates from *Bocconia microcarpa* (Maxim.) Fedde[58]. Chelerythrine nitrate was precipitated with nitric acid from the aqueous filtrate after processing the *Fagara semiarticulata* bark[59]. Hydrochloric acid is often used for the separation of quaternary benzo[*c*]phenanthridine alkaloids (e.g. from *Ch. majus*[60], *D. lactucoides*[38]). The precipitate of nitidine chloride (*7*) was obtained from the chloroform extract after addition of 1M hydrochloric acid[61] (*Zanthoxylum flavum*

VAHL). The precipitate of sanguinarine resulted by introducing dry gaseous hydrogen chloride into the benzene extract from *Argemone mexicana* seeds[62]. Recently the isolation of QBPAs from *S. canadensis* has been patented using methanol acidulated with hydrochloric and citric acid and a successive precipitation with zinc chloride[63].

(ii) The QBPAs can be separated from the mixture of alkaloids as insoluble 6-cyano-5,6-dihydro derivatives (pseudocyanides) by treatment with an excess of potassium cyanide[41,64]. Pseudocyanides can be converted to quaternary chlorides by reflux in chloroform–methanol solution with hydrochloric acid[65].

(iii) In plant species with relatively low content of QBPAs the preparative liquid column chromatography is a very useful isolating method. An extract is carried on an adsorbent (mostly silica or alumina) and is eluted with solvents with gradually increasing polarity. Thus, sanguinarine (*1*) and chelerythrine (*2*) were isolated from *Pteridophyllum racemosum* SIEB. et ZUCC.[66], chelerythrine (*2*) and nitidine (*7*) from *Zanthoxylum Williamsi*[67] and *Fagara viridis* A. CHEVAL[68]. Nitidine was obtained by preparative chromatography on Sephadex LH-20 in methanol–chloroform (3 : 1) from an extract of *Fagara macrophylla* ENGL.[69]. Sanguinarine and chelery-

TABLE V
The chronological survey of isolation of quaternary benzo[*c*]phenanthridine alkaloids

| Alkaloid | The first isolation | Determination of structure |
|---|---|---|
| Sanguinarine (*1*)[a] | 1828, Dana[40]<br>1924, Gadamer[41] | 1931, Späth[42] |
| Chelerythrine (*2*)[a] | 1839, Probst[43]<br>1893, König[44] | 1931, Späth[45] |
| Chelirubine (*3*) | 1954, Slavík[46] | 1975, Ishii[47] |
| Chelilutine (*4*) | 1954, Slavík[46] | 1977, Ishii[48] |
| Macarpine (*5*) | 1955, Slavík[49] | 1981, Takao[50] |
| Avicine (*6*) | 1959, Arthur[51] | 1959, Arthur[51] |
| Nitidine (*7*) | 1959, Arthur[52] | 1959, Arthur[52] |
| Sanguirubine (*8*) | 1960, Slavík[28] | 1978, Ishii[53] |
| Sanguilutine (*9*) | 1960, Slavík[28] | 1978, Ishii[53] |
| Fagaronine (*10*) | 1972, Messmer[54] | 1972, Messmer[54] |
| Fagaridine (*11*) | 1973, Torto[55] | 1973, Torto[55] |
| Puctatine (*12*) | 1977, Stermitz[56] | 1977, Stermitz[56] |
| 7,8-Demethylene-sanguinarine (*13*)[b] | 1978, Lasskaya[57] | 1978, Lasskaya[57] |
| 7,8-Demethylene--7,8-dehydro-sanguinarine (*14*)[b] | 1978, Lasskaya[57] | 1978, Lasskaya[57] |

[a] Isolated as a sum of alkaloids; [b] probably an artifact.

thrine were isolated as pseudoalcoholates by separating the extract from *Hunnemania fumariaefolia* SWEET on silica[70]. The use of preparative column chromatography allows at the same time to separate individual alkaloids. For a detailed survey of isolating methods see refs[27,65].

### 4.2. SEPARATION

The separation of individual quaternary benzo[*c*]phenanthridines from each other is rather difficult due to their considerable chemical similarity. The differences in solubility of various forms of alkaloids were used to the preliminary division. However, such a manner is suitable in only those cases when the mixture of QBPAs is not too complicated.

Chelerythrine was obtained by repeated crystallization of the pseudocyanide mixture from acetone[45,64]. Sanguinarine and chelerythrine can be separated from each other by repeated crystallization of their tartrates from water[41]. The separation of both alkaloids is also possible via manyfold crystallization of their nitrates from water. Sanguinarine nitrate precipitates from the solution the majority of chelerythrine remains in mother liquor[45].

The better solubility of chelerythrine chloride in chloroform[71] and the poor solubility of chelerythrine pseudobase in ether[58] were also employed to separate sanguinarine from chelerythrine.

The separation of QBPAs from each other was reached by preparative column chromatography using acidic alumina. The eluting system was the mixture of benzene saturated with water–acetic acid (99 : 1) with a gradually increasing amount of ethanol[46]. In this way two minor alkaloids chelirubine (*3*) and chelilutine (*4*) were discovered in *Chelidonium majus*[46] and two further alkaloids sanguirubine (*8*) and sanguilutine (*9*) in *Sanguinaria canadensis*[28] and macarpine (*5*) in *Macleaya microcarpa* (MAXIM.) FEDDE[49].

The preparative TLC permits one to obtain miligramme amounts of sanguinarine and chelerythrine sufficient for spectral identification[72]. The countercurrent cascade extraction of *Chelidonium majus* alkaloids has been reported[73]. The preparative centrifugal TLC served for the separation and purification of benzo[*c*]phenanthridine alkaloids isolated from *Zanthoxylum spinosum* (L.) Sw.[74].

### 4.3. QUANTITATIVE DETERMINATION

Various methods of determination of QBPAs in all kinds of materials have been reported.

The spectrophotometric determination of sanguinarine (*1*) and chelerythrine (*2*) in *Bocconia cordata* extract[75] and in the pharmaceutical preparation Sangviritrin[76] has been described. After TLC division the zones of *1* and *2* were mechanically

removed. Alkaloids were extracted with chloroform as the complexes with tropeoline 000–2. Absorbance of the chloroform solution was measured at 410–420 nm. In the same manner the calibration curves were made[75,76]. A similar method was used for the determination of sanguinarine and chelerythrine from *Chelidonium majus*[77]. Chelerythrine was determined at 234 nm, sanguinarine at 286 nm.

Sanguinarine was determined in seed oil of *Argemone mexicana* L. as well as in edible oils. The investigated material was dissolved in petroleum ether and the present sanguinarine was reduced with sodium borohydride to dihydrosanguinarine (*63*). This derivative was irradiated with UV light on a TLC plate in order to convert it to sanguinarine. After extraction from the TLC plate the absorbance of sanguinarine was measured at 330 nm. The concentration was read from the calibration curve[78].

Freytag[79] developed the method for quantitative determination of chelidonine, chelerythrine and sanguinarine by direct remission measuring of HPTLC plates. This method is recommended for analyses of pharmaceutical raw materials Herba chelidonii, Radix chelidonii and Tinctura chelidonii.

Capillary isotachophoresis was used for qualitative and quantitative analyses of some quaternary isoquinoline alkaloids (including sanguinarine). It allows sanguinarine and chelerythrine to be determined in a crude extract in the presence of other alkaloids[80].

There are also several papers on the determination of QBPAs in various materials by HPLC.

Dried rhizomes of *Sanguinaria canadensis*, sanguinaria extract and commercial toothpaste and oral-rinse (Viadent) were analysed in order to find out the content of QBPAs. The method was specified as a quality checking technique for oral health care products[39]. Further human saliva and plaques collected after rinsing with sanguinaria oral rinse were analysed by HPLC employing methanol–water (84 : 16) with triethylamine and phosphoric acid as a mobile phase[39,81].

A simple, rapid and reproducible HPLC method to determine *1* and *2* in plant extract was published[35]. The mobile phase was a solution of sodium acetate in methanol with 1,4-dioxan and acetic acid. The content of sanguinarine (*1*) and chelerythrine (*2*) was found to be 0·38% and 0·33%, resp., in *Chelidonium majus* root. The use of reversed-phase or ion pair chromatography gave unsatisfactory results[35].

On the other hand, Freytag[60] described HPLC analysis of chelidonine, sanguinarine and chelerythrine isolated from *Chelidonium majus*. He used normal phase (eluent: toluene with methanol), reversed-phase and ion pair chromatography (eluent: methanol containing triethanolamine and sodium hydrogenphosphate). Contents of alkaloidal cells in *Macleaya cordata* root and *Sanguinaria canadensis* rhizomes were analysed quantitatively by histochemical chromatography[82]. The total content of sanguinarine and chelerythrine (as chlorides) was found to be 1–6 ng per single

cell in *M. cordata*. In *S. canadensis* the content of both alkaloids was considerably higher: 137 ng sanguinarine and 68 ng chelerythrine per single cell[82].

The rapid HPLC determination of argemone oil in edible groundnut and mustard oil is based on the appearance of the peak of sanguinarine[83]. Sanguinarine is a regular component of argemone oil which is indesirable in edible oils.

Recently the differential pulse polarography has been used for the determination of total QBPAs in sanguinaria extract-based oral rinses[84].

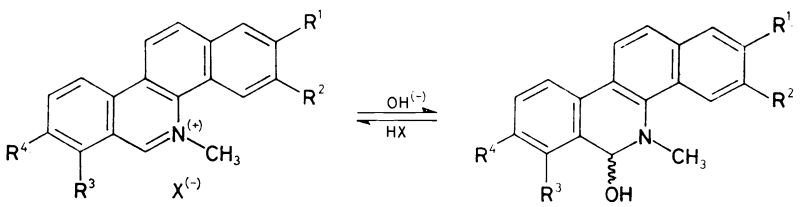
## 5. CHEMICAL REACTIVITY

The reactivity of quaternary benzo[*c*]phenanthridinium cations is characterized by the sensitivity of the polar bond N(5)=C(6) to the attack of nucleophiles. This statement is supported by the data given in literature which deal with synthetic preparation of derivatives, isolation of artifacts, so called pseudobase formation or an interaction of QBPAs with biopolymers.

Also the quantum chemistry calculations show that the lowest $\pi$-electron density is located in position 6. The Fig. 3 shows the distribution of $\pi$-electron density in the molecule of phenathridine calculated[85] by SCF ASMO CI and the Fig. 4 in sanguinarine (*1*) calculated by LCAO method[86].

### 5.1. Pseudobase Formation

The data in the literature based on spectral measurements talk about an equilibrium between the quaternary cation and the adduct of hydroxide anion to the polar bond N(5)=C(6) which is called pseudobase (synonyms: base, alkanolamine or carbinol form) (Scheme 1). The equilibrium is pH dependent.




Scheme 1

The name of pseudobase came from the last century[87]. The pseudobase formation seems to be a general phenomenon of the molecules of heterocyclic cations[88]. Therefore the formation of pseudobase from N-substituted quinolinium, isoquinolinium and phenanthridinium cations was the object of an interest of many authors (cf. comprehensive reviews[88,89]).

The equilibrium between heterocyclic cation ($Q^+$) and the corresponding pseudobase (QOH) may be formulated as a complex formation

$$Q^+ + OH^- \rightleftarrows QOH \qquad (A)$$

and the constant of association for this equilibrium

$$K = [QOH]/[Q^+][OH^-] \qquad (B)$$

or more often as acidobasic equilibrium:

$$Q^+ + H_2O \rightleftarrows QOH + H^+ \qquad (C)$$

with the constant

$$K_{R+} = [H^+][QOH]/[Q^+]. \qquad (D)$$

The $pK_{R+}$ value which is analogous to the $pK_a$ value for a Brønsted acid denotes the pH at which the heterocyclic cation and pseudobase are present at the equal concentrations.

The values $pK_{R+}$ of QBPAs were mostly determined by spectrophotometry, sometimes by fluorometry or potentiometry and are given in Table VI.

From Table VI we can deduce that sanguinarine forms the pseudobase at lower pH than chelerythrine and is more sensitive to the attack of $OH^-$ anion. The influence of detergents upon the pseudobase formation was also followed. A cation-active detergent makes sanguinarine and chelerythrine more acidic[93]. From the last measurements[94], using UV/HPLC method, the iminium form of sanguinarine (*I*) is the prevailing form under pH 5·4. The conversion to the alkanolamine (pseudobase) is nearly complete at pH 7 (about 5% of iminium form is present only). At pH 9·8 less than 1% of iminium form is present in the buffer solutions with 20% of ethanol[94].

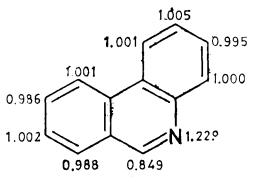



FIG. 3
π-Electron density in phenanthridine

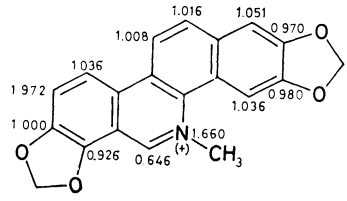



FIG. 4
π-Electron density in sanguinarine (*I*)

The method of preparation of the pseudobase from sanguinarine is given in refs[28,34,95] and from chelerythrine in refs[28,95,96]. The quaternary alkaloid is dissolved in water and the solution made alkaline with NaOH or ammonia and then so prepared stuff extracted with ether or benzene. After concentration of the solvent the product crystallizes out.

In the ref.[95] the yield of pseudobase from *1* is reported to be 64% and from *2* 66%. But the given $^1$H NMR spectral data do not prove the presence of OH group and IR spectral data of both pseudobases are not given. The NMR spectrum of sanguinarine pseudobase in hexadeuteriodimethyl sulfoxide shows a broad singlet at 5·70 ppm belonging to the hydrogen atom bound at C(6). Any other signal which might be the signal of OH is missing. Therefore it shows on the presence of some kind of pseudoderivative only.

After addition of trifluoroacetic acid to the solution of pseudobase the conversion to the iminium form is observed. Whatever group at C(6) through oxygen is bound, such a conversion takes place.

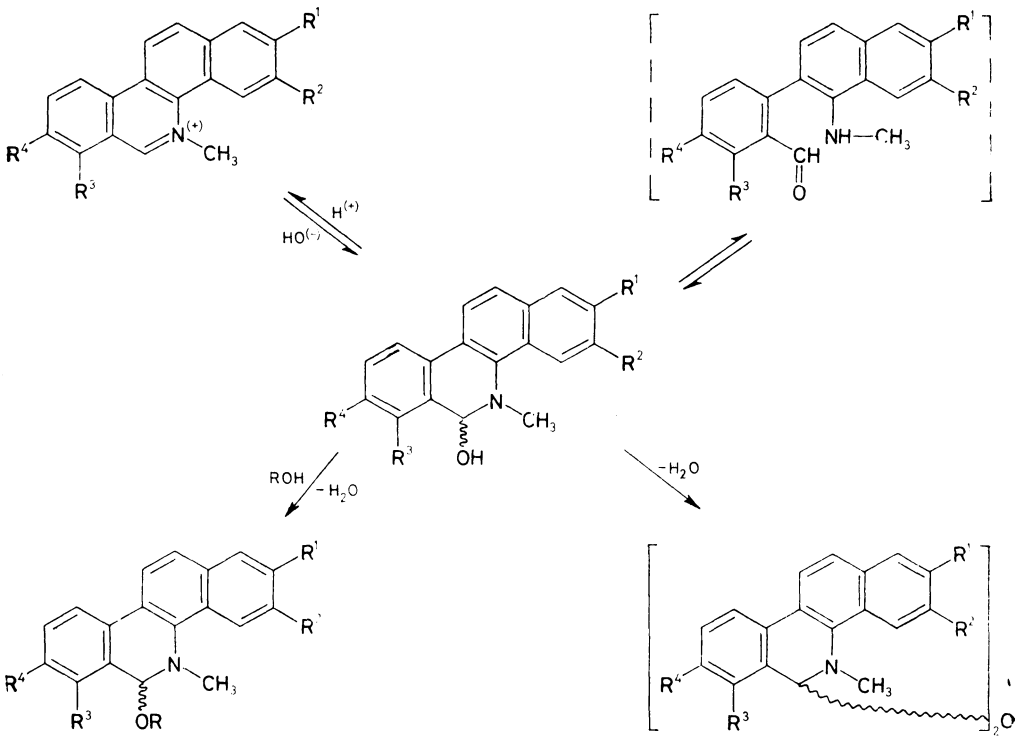
According to the ref.[96] the material extracted from alkaline solution of *2* (called as chelerythrine-base) is a mixture of two compounds with character of tertiary amine. Its $^1$H NMR spectrum is published but the signal of the OH group in it is again missing. The mass spectrum shows peaks characteristic for chelerythrine and moreover some very small peaks responding to ion $M^+$, $(M - H)^+$ and $(M - 2H)^+$ derived from pseudobase form. Authors[96] came to the following unclearly defined conclusion that pseudobase cannot be described because different 6-substituted products are being obtained according to reaction conditions.

There are still some doubts whether so-called pseudobase derived from a quaternary benzo[*c*]phenanthridine alkaloids is a compound with a free hydroxy group as it is frequently mentioned in literature or if it is so-called bimolecular ether (anhydrobase)[28,34,97] or another equivalent of the double molecular weight (Scheme 2).

TABLE VI
Values of $pK_{R+}$ for sanguinarine and chelerythrine in water

| Alkaloid | Spectroscopy | | Fluorometry | | Potentiometry ref.[92] |
|---|---|---|---|---|---|
| | ref.[90] | ref.[91] | ref.[90] | ref.[91] | |
| Sanguinarine (*1*) | 8·05 | 5·75[a] | 7·95 | 7·92 | 7·32 |
| Chelerythrine (*2*) | 9·00 | 6·67[a] | 8·90 | 8·77 | 7·53 |

[a] In water–ethanol (1 : 1).



SCHEME 2

Salts of quaternary benzo[*c*]phenanthridine alkaloids either in a crystalline form or in aqueous or alcoholic solutions are characterized by the significant colouring for instance sanguinarine (*1*) is copper red, chelerythrine (*2*) yellow, chelirubine (*3*) dark purple, chelilutine (*4*) yellowish-orange, macarpine (*5*) and sanguirubine (*8*) purple red, sanguilutine (*9*) golden-yellow, fagaronine (*10*) and fagaridine (*11*) yellow etc.

### 5.2. Addition of O-Nucleophiles

In addition to $OH^-$ anion alcoxide anions and alcohols are the other O-nucleophiles whose reactions were followed.

In a buffer (pH 7) with 25% of ethanol 14% of sanguinarine iminium form was found and in the same mixture 51% of chelerythrine iminium form was found. In a higher concentration (50%) of ethanol the only 3% of quaternary sanguinarine and 10% of quaternary chelerythrine were found, respectively[26]. This indicates that the majority of the alkaloid is present in the form of the adduct with alcohol (pseudo-

alcoholate). It also means that sanguinarine is more sensitive against the attack of alcohol.

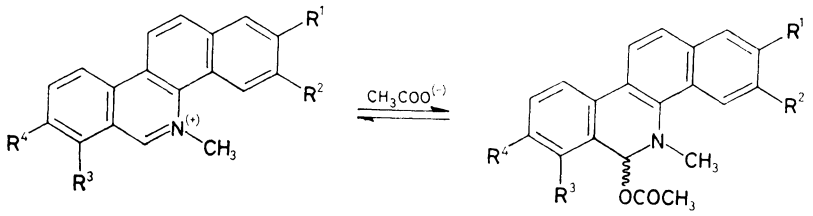
Tolkachev and coworkers[98] studied the formation of 6-ethoxy-5,6-dihydro derivatives (pseudoethanolates) of sanguinarine and chelerytrine during the crystallization of their quaternary salts from 48% ethanolic solution using NMR, TLC and potentiometric titration. After a 3 times repeated crystallization of the mixture of hydrogensulfates of sanguinarine and chelerythrine from 48% ethanol the mixture contained about 25% of an untitrationable stuff which they considered as the pseudoalcoholate. Their conclusions are following: Sanguinarine is more reactive against alcohol than chelerythrine due to a better interaction of electron pairs of oxygen atoms of the rigid dioxolane ring of sanguinarine molecule with $\pi$-electron orbitals of aromatic rings C and D than that of free rotating methoxy groups in the molecule of chelerythrine. This results in the different electron density on C(6) where the nucleophilic attack proceeds and consequently in the difference in the reactivity.

6-Ethoxy-5,6-dihydrochelerythrine (*50*) was prepared by the several times repeated crystallization of the chelerythrine pseudobase form absolute ethanol[28,59]. Sanguinarine pseudomethanolate (*51*) was prepared by the reaction of sanguinarine chloride with an excess of sodium methanolate in dichloromethane[70] or by the crystallization of pseudobase from chloroform–methanol solution[28,99].

In the literature preparations of 6-propoxy- and 6-butoxy-5,6-dihydro derivatives of sanguinarine *52*, *53* and the same derivatives of chelerythrine[100] as well as 6-benzyloxy-5,6-dihydrosanguinarine[34] (*54*) are also reported.

The method of preparation of 6-methoxy-5,6-dihydronitidine (*55*) has lately been described[69]: the suspension of nitidine chloride (*7*) in concentrated ammonia was stirred at room temperature and then extracted with ethyl acetate. After the solvent evaporation a syrupy material was obtained and this was recrystallized from methanol with the yield of 81% of crystalline pseudomethanolate *55*.

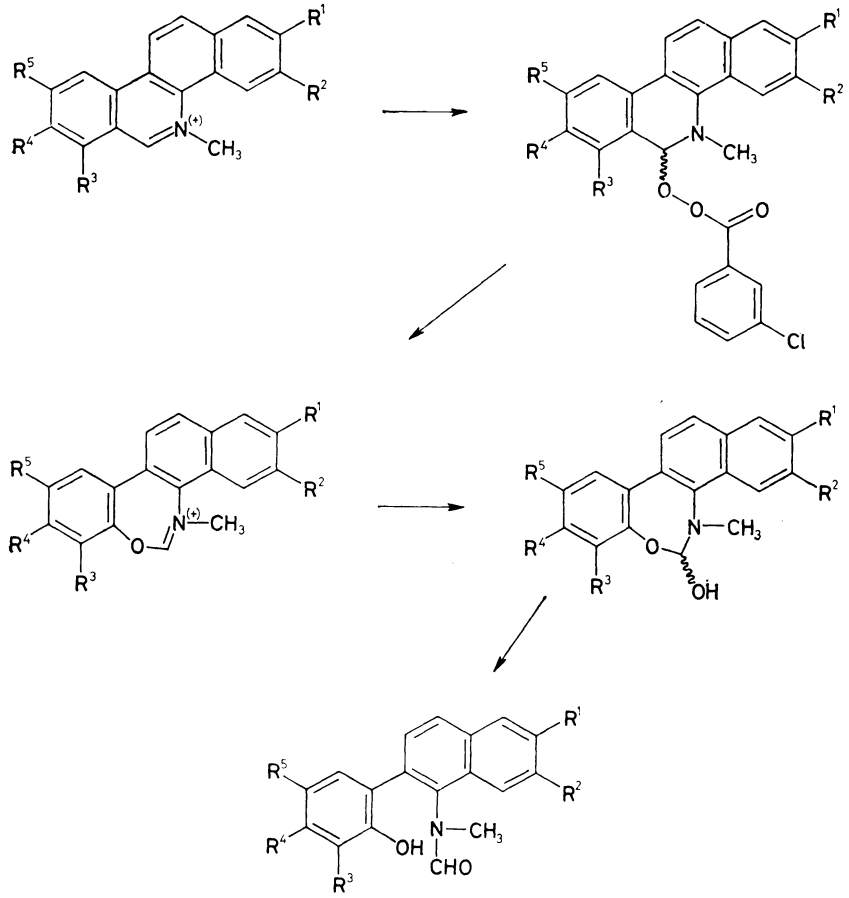
The study of the equilibriums in the solutions of sanguinarine and chelerythrine acetates in dependence on the polarity of the used solvent is very interesting. NMR spectroscopy of the solution of chelerythrine acetate in deuterooxide indicates the presence of the iminium form only (Scheme 3). In the chloroform solution one can notice the presence of 2 substances. The authors suppose the presence of pseudo-




SCHEME 3

acetate and its open amino-aldehyde form which is proved by the presence of the signal with the chemical shift of aldehyde proton at 9·92 ppm and by the vibrations at 1 710 and 1 755 $cm^{-1}$ in IR spectrum. But no formula of the mentioned aldehyde form is given[101].

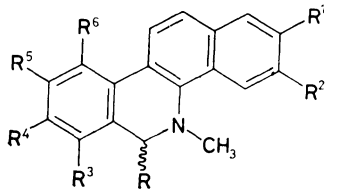
Baeyer–Villiger reaction of quaternary benzo[*c*]phenanthridine with *m*-chloroperoxybenzoic acid in HMPA is described. The primary formed adduct cyclizes to 1,3-oxazepine derivative which undergoes the hydrolysis to secobenzo[*c*]phenthridine (Scheme 4). Under the same conditions chelerythridine chloride (*2*) gives arnottianamide (*56*) in the 70% yield[102]. Analogously avicine (*6*) gives integriamide (*57*)




*56*, $R^1 + R^2 = OCH_2O$ ; $R^3 = R^4 = OCH_3$ ; $R^5 = H$
*57*, $R^1 + R^2 = R^4 + R^5 = OCH_2O$ ; $R^3 = H$
*58*, $R^1 + R^2 = OCH_2O$ ; $R^3 = H$ ; $R^4 = R^5 = OCH_3$

SCHEME 4

in the 79% yield[103] and nitidine (*7*) isoarnottianamide (*58*) in the yield[102] of 27%.




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $R^6$ | R |
|---|---|---|---|---|---|---|---|
| *50* | $OCH_2O$ | | OMe | OMe | H | H | OEt |
| *51* | $OCH_2O$ | | $OCH_2O$ | | H | H | OMe |
| *52* | $OCH_2O$ | | $OCH_2O$ | | H | H | $O(CH_2)_2CH_3$ |
| *53* | $OCH_2O$ | | $OCH_2O$ | | H | H | $O(CH_2)_3CH_3$ |
| *54* | $OCH_2O$ | | $OCH_2O$ | | H | H | OBz |
| *55* | $OCH_2O$ | | H | OMe | OMe | H | OMe |
| *59* | $OCH_2O$ | | $OCH_2O$ | | H | H | $NHC_6H_5$ |
| *60* | $OCH_2O$ | | $OCH_2O$ | | H | H | $NHNHC_6H_5$ |
| *62* | OH | OMe | H | OMe | OMe | H | H |
| *63* | $OCH_2O$ | | $OCH_2O$ | | H | H | H |
| *64* | $OCH_2O$ | | $OCH_2O$ | | H | OMe | H |
| *65* | $OCH_2O$ | | OMe | OMe | H | OMe | H |
| *66* | OMe | OMe | OMe | OMe | H | OMe | H |
| *67* | $OCH_2O$ | | OMe | OMe | H | H | CN |
| *68* | $OCH_2O$ | | $OCH_2O$ | | H | H | CN |
| *69* | $OCH_2O$ | | OMe | OMe | H | H | Me |
| *70* | $OCH_2O$ | | OMe | OMe | H | H | Et |
| *71* | $OCH_2O$ | | OMe | OMe | H | H | $C_6H_5$ |
| *72* | $OCH_2O$ | | $OCH_2O$ | | H | H | $CH_2NO_2$ |
| *73* | $OCH_2O$ | | $OCH_2O$ | | H | H | $CH_2COOH$ |
| *74* | $OCH_2O$ | | OMe | OMe | H | H | $CH_2COCH_2CH_3$ |
| *75* | $OCH_2O$ | | OMe | OMe | H | H | $CH_2COH$ |
| *76* | $OCH_2O$ | | OMe | OMe | H | H | $CH_2COCH_3$ |
| *79* | $OCH_2O$ | | OMe | OMe | H | H | $CH_2OH$ |
| *80* | $OCH_2O$ | | OMe | OMe | H | H | $CH_2COCH_2CH(CH_3)_2$ |
| *81* | $OCH_2O$ | | OMe | OMe | H | H | X |
| *83* | $OCH_2O$ | | H | OMe | OMe | H | H |
| *85* | $OCH_2O$ | | H | $OCH_2O$ | | H | H |

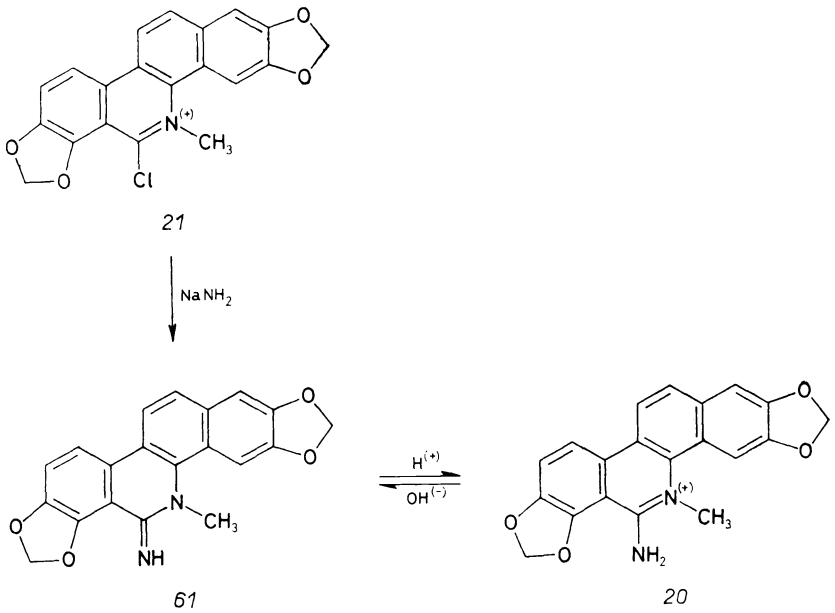
Me methyl, Et ethyl, Bz benzyl, X 2-oxo-3-pyrrolidinyl

### 5.3. Addition of N-Nucleophiles

Karrer[104] described the reaction of chelerythrine with phenylhydrazine and because the structure of the starting compound was not known at that time he assumed a reaction with the carbonyl group.

6-Anilino- and 6-phenylhydrazino-5,6-dihydrosanguinarine were prepared by Beke[34]. The products are characterized by the elemental analysis only and no spectral data are given. Their structures are represented by the formulas *59* and *60*. By the hydrogenation of these adducts on palladium dihydrosanguinarine (*63*) was obtained in the yield of 70–80%.

In the paper[21] the reaction of 6-chlorosanguinarine (*21*) with sodium amide in ether giving 6-iminosanguinarine (*61*) in the yield of 69% (Scheme 5) is mentioned.




SCHEME 5

This derivative under treatment of acid undergoes into the quaternary salt of 6-aminosanguinarine (*20*). 6-Aminosanguinarine was isolated as artifact from *Glaucium flavum Cr.* var. *vestitum*. 6-Chlorosanguinarine (*21*) was obtained by the reaction of 6-oxosanguinarine (*87*) with phosphorus oxychloride. Any attempts to get 6-iminosanguinarine from the reaction of sanguinarine with ammonia under varied conditions failed[21].

### 5.4. Addition of S-Nucleophiles

In the literature there is only little information about the addition of S-nucleophiles to QBPAs. It seems that this field was not a target of interest from the preparative

point of view. The addition of –SH groups is assumed to play a role in the interaction of enzymes with QBPAs.

Sarkar[105] found that sanguinarine at pH 7·4 after addition of 2,3-dimercaptopropanol (BAL) or monoethyleneglycol, in the molar ratio alkaloid–thiol = 1 : 1, forms a precipitation and the solution after filtration shows the negative reaction on the presence of sulfur.

It was found[106] that compounds with –SH group protect enzyme alanine-amino--transferase (ALT) against the inhibition influence of sanguinarine or chelerythrine. Again the addition of thiol group to the polar iminium bond of QBPA is supposed. The constants of stability $K$ of the adducts of QBPAs with thioethanol, cysteine and glutathione were established from the molar absorptivity at the wavelength where the absorbance of QBPAs differs from the absorbance of the adduct.

$$A + S \rightleftarrows AS \qquad (E)$$

$$K = [AS]/[A] \,.\, [S] \,, \qquad (F)$$

where [A] is the concentration of alkaloid, [S] concentration of thiol, [AS] concentration of adduct.

Glutathione that has the highest constant of the stability is the most effective against the inhibition activity of both alkaloids (Table VII).

### 5.5. Addition of H-Nucleophile

Complex hydrides in the reaction with QBPAs give 5,6-dihydro derivatives in a good yield. Thus, fagaronine chloride (*10*) by the reaction with sodium borohydride in methanol at room temperature after 1 h gives 5,6-dihydrofagaronine (*62*) in the yield[25] of 82%.

Table VII

Stability constants of adduct formation (log $K$) for sanguinarine and chelerythrine with some thiols (data from ref.[106])

| Thiol | Sanguinarine (*1*) (pH 5·28) | Chelerythrine (*2*) (pH 5·68) |
|---|---|---|
| Thioethanol | 3·77 | 3·46 |
| Cysteine | 2·97 | 2·74 |
| Glutathione | 4·64 | 4·39 |

The reduction of crude commercial sanguinarine (*1*) with sodium borohydride and subsequent oxidation of the product is one of the methods of purification of sanguinarine[107]. Commercial sanguinarine nitrate, which contains chelerythrine and protopine, was treated with sodium borohydride in methanol at room temperature. After the reaction the solvent was evaporated and the rest extracted with chloroform and purified by column chromatography. The pure dihydrosanguinarine (*63*) was then oxidized to *1* with mercury acetate.

The information that QBPAs may be reduced[108] with coenzymes NADH and NADPH to their 5,6-dihydro derivatives seems to be very interesting. The reaction is reversible in the stoichiometry 1 : 1. These experiments show a possible role of the redox reactions because dihydrobenzo[*c*]phenanthridines are there found together with QBPAs as it was discovered for example in *Chelidonium majus* L. (ref.[109]).

Dihydrosanguilutine (*66*) was oxidized to aromatic sanguilutine chloride (*9*) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in benzene at room temperature[110] (yield 92%).

A number of minor alkaloids as chelirubine (*3*), chelilutine (*5*) and sanguilutine (*9*) was treated with sodium borohydride in the mixture of water and methanol at room temperature[109]. The corresponding dihydroderivatives *64*, *65* and *66* were obtained in high yields. However, their spots on TLC plates were very quickly oxidized by oxygen to the starting intensively coloured quaternary alkaloids.

### 5.6. Addition of C-Nucleophiles

In 1917 Karrer[104] described the reaction of chelerythrine (*2*) with KCN. To the aqueous solution of *2* cyanide was added. The original yellow colour of the solution changed and the white precipitate was formed. This compound called pseudocyanide *67* is characterized as a nonbasic compound insoluble in water. Its treatment with boiling concentrated acid gives again the starting material that is chelerythrine (*2*).

Similarly Gadamer[97] prepared sanguinarine pseudocyanide (*68*); its properties are very similar to *67*. The addition of silver nitrate caused the precipitation of silver cyanide.

The formation of pseudocyanide served as the method of preparation of the pure chelerythrine and determination of the structure[45]. From the commercial product of Merck the mixture of pseudocyanides of *1* and *2* was obtained. The pure chelerythrine pseudocyanide (*67*) was separated from pseudocyanide of sanguinarine (*68*) by the repeated crystallization from acetone. The compound thus formed was then treated with boiling acid forming quaternary salt *2*.

The formation of the pseudocyanides (5-cyano-5,6-dihydro derivatives) insoluble in water is very often used for the isolation of QBPAs from the other tertiary alkaloids[28,32,46,65].

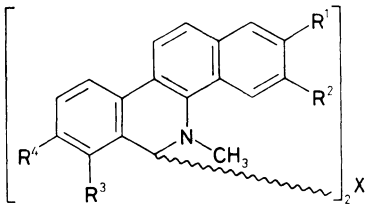
Pseudocyanides serve as well as 6-oxo and 5,6-dihydro derivatives for the characterization[20,24] of the synthesized QBPAs.

A rather curious method of pseudocyanide preparation is described in ref.[59]. 6-Ethoxy-5,6-dihydrochelerythrine (*50*) was dissolved in the mixture chloroform–methanol and heated. After heating which was probably needed for the quaternary cation formation the aqueous solution of KCN was added and the mixture refluxed for 15 min. Then the pseudocyanide *67* was isolated.

The reaction of QBPAs with the Grignard reagent was for the first time mentioned by Karrer[104]. He prepared methyl, ethyl, and propyl derivatives by the reaction of chelerythrine with alkylmagnesiumiodides. The products were called chelalbine – white crystalline compounds of nonbasic properties. Gadamer[41] designated them correctly as alkyldihydrochelerythrines. The reaction of chelerythrine chloride and the Grignard reagent was carried out in boiling ether as long as the yellow colour disappeared. Then hydrochloric acid was added and the product filtered off and crystallized. Thus were prepared 6-methyl- (*69*), 6-ethyl- (*70*) and 6-phenyl-5,6-dihydrochelerythrine (*71*).

In the paper[34] the formation of 6-nitromethyl-5,6-dihydrochelerythrine (*72*) (80% yield) and 6-(5,6-dihydrosanguinarinyl)acetic acid (*73*) (50% yield) are mentioned. The structure of the given formulas is not supported by any spectral data.

In the presence of concentrated solution of sodium carbonate chelerythrine chloride enters into the reaction with 2-butanone (6 h at 80°C) forming 6-propionylmethyl-5,6-dihydrochelerythrine (*74*). This synthesis supported the structure of alkaloid primarily isolated[111] from *Fagara mayu* (BERT. ex HOOK. et ARN.) ENGLER (*Rutaceae*).




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | X |
|---|---|---|---|---|---|
| *77* | $OCH_2O$ | | OMe | OMe | $CH_2COCH_2$ |
| *78* | $OCH_2O$ | | $OCH_2O$ | | $CH_2COCH_2$ |
| *82* | $OCH_2O$ | | OMe | OMe | CH(COH) |

Also the reaction of chelerythrine pseudobase with acetaldehyde[96] at room temperature (3 h) is an example of the reaction with C-nucleophiles. As the product 6-formylmethyl-5,6-dihydrochelerythrine (*75*) was identified.

Reflux of chelerythrine chloride with acetone in the presence of aqueous solution of sodium carbonate for several hours gives 6-acetonyl-5,6-dihydrochelerythrine (*76*) (ref.[113]). The analogous conversion of sanguinarine was reported in ref.[112]. But our experience shows that this reaction is instantaneous.

The reaction of chelerythrine pseudobase with the mixture of acetone and methanol (2 : 1) refluxing gave adduct *76* and dimeric derivative *77* called chelerythridimerine[96]. The latter compound *77* can also be obtained by the reaction of chelerythrine with 3-oxo-1,5-pentanedioic acid in pyridine following MacLean procedure[113].

Analogously 1,3-bis-(6-dihydrosanguinarinyl)-acetone (chelidimerine) (*78*) was obtained and in this way the structure of the alkaloid isolated from *Chelidonium majus* L. was confirmed (ref.[114]).

An optically active alkaloid of the same constitution – sanguidimerine – was isolated from *Sanguinaria canadensis* L. (ref.[115]).

UV light causes the chemical reaction of chelerythrine chloride in the mixture methanol and acetone leading to 6-hydroxymethyl-5,6-dihydrochelerythrine (bocconoline) (*79*) in 60% yield after 105 min exposition time[116].

Pseudoderivatives of quaternary benzo[*c*]phenanthridine alkaloids with a carbon substituent bound at C(6) were often succeeded to isolate from plants.

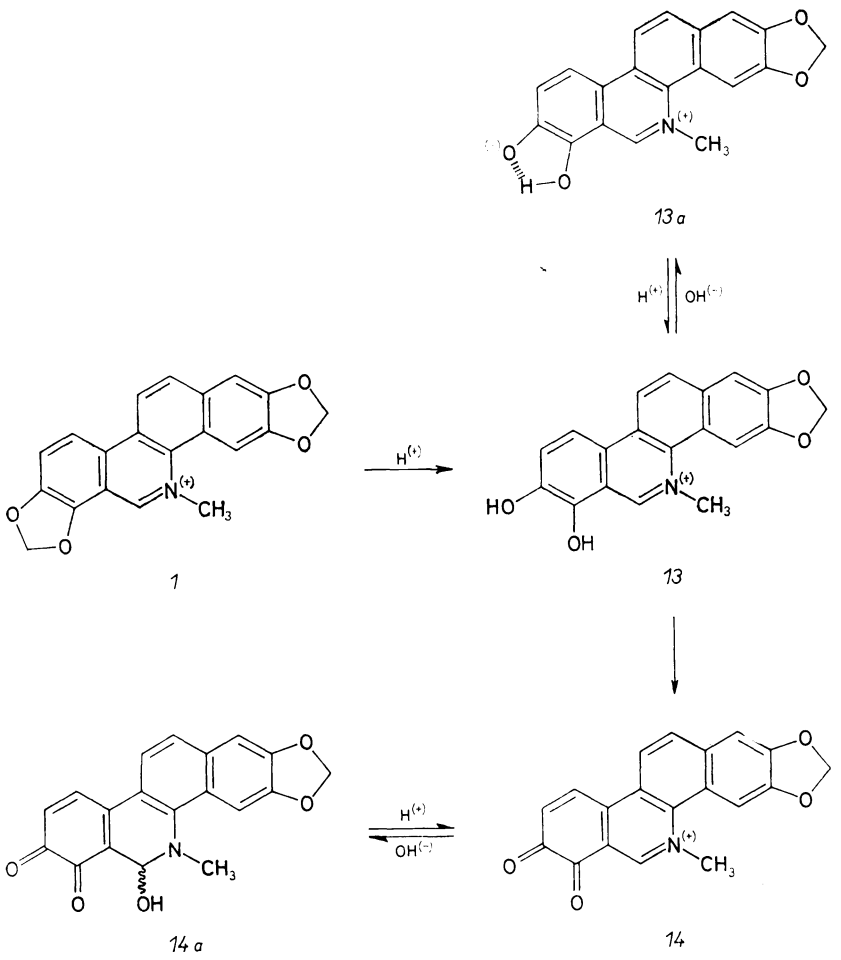
For instance, lately the isolation of several new benzophenanthridine alkaloids from two Zanthoxylum species has been reported. These are derivatives of dihydrochelerythrine with a carbon substituent in position 6. Besides the mentioned derivatives 6-(4-methyl-2-oxopentanyl)dihydrochelerythrine (80), 6-[3′-(2-oxopyrrolidinyl)] dihydrochelerythrine (chelactam) (*81*) and dimeric alkaloid 2,2-bis-[6′-(dihydrochelerythrinyl)] acetaldehyd (*82*), so called caymandimerine, were isolated[74].

These isolated substances are considered to be artifacts according to some authors[4,21,112].

### 5.7. Demethylenation of Sanguinarine[57]

The reaction leading to the demethylenation is carried out in acid. Thus, when the mixture of sanguinarine (*1*) and chelerythrine (*2*) hydrogensulfates was heated in 5% sulfuric acid for 4 h and then neutralized with ammonia and extracted with chloroform, the chloroform extract of several compounds was obtained. The concentrated extract underwent the chromatographic separation on the column of aluminium oxide with chloroform as eluent. The upper zone (violet colour) was mechanically separated and gave 7,8-demethylensanguinarine (*13*) as crystalline trifluoroacetate. The other zone gave 7,8-demethylene-7,8-dehydrosanguinarine (*14*). This according to the authors forms in the alkaline medium pseudobase *14a*. But no spectral evidence of this structure was given. The structure of both derivatives *13* and *14* was established by NMR spectroscopy. The demethylenated sanguinarine (*13*) showed the tendency to tautomeric equilibrium under formation of amfion *13a*.

Phenolic hydroxy groups of *13* very easily undergo the oxidation under formation of *o*-quinoid structure *14* (Scheme 6). Both compounds were isolated as minor components accompaning sanguinarine and chelerythrine in the plant species *Macleaya cordata* (WILD.) R.BR. and *M. microcarpa* (MAXIM.) FEDDE.




SCHEME 6

Chelerythrine (*2*) under conditions of the reaction did not enter into any reaction. The different reactivity of sanguinarine and chelerythrine may be explained by the higher sensitivity of dioxolane ring in the position 7,8- against acid catalyzed hydrolysis due to the presence of the electron-withdrawing group $-CH=N^{(+)}-CH_3$.

Compound *13* is known as artifact formed during isolation and present in the longer time kept samples of sanguinarine. Its amount according to authors usually does not exceed 1–2%.

## 5.8. Redox Reactions

### *Reduction*

Fully aromatic quaternary benzo[*c*]phenanthridinium cation can undergo the reduction with zinc powder[59,69,104] in diluted hydrochloric acid under reflux (few hours). The result is the same as after addition of hydride anion (Chapter 5.5.). The corresponding 5,6-dihydrobenzo[*c*]phenanthridine alkaloid is formed.

### *Disproportionation*

The two of QBPAs – avicine (*6*) and nitidine (*7*) differ in their behaviour in alkaline medium from the other QBPAs. This was discovered during their isolation[51,52]. Thus, 5,6-dihydronitidine (*83*) and 6-oxonitidine (*84*) were obtained by chromatography on alumina under argon following procedure: *Zanthoxylum nitidum* (Lam.) DC. root bark was extracted with hot methanol then concentrated and extracted again with hot water. Basification of the aqueous extract with ammonia gave a buff-colored precipitate which was chromatographed.

Similar behaviour showed also alkaloid avicine isolated from *Zanthoxylum avicennae* (Lam.) DC. The crude quaternary salt of avicine isolated from methanolic extract could not be purified by the common method i.e. conversion to pseudobase and subsequent reconversion to quaternary salt, because after basification of the solution 5,6-dihydroavicine (*85*) and 6-oxoavicine (*86*) were found.

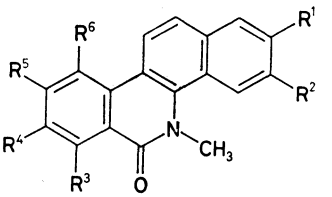
Dihydroavicine (*85*) and dihydronitidine (*83*) are said not to be very stable and undergo in the presence of oxygen an oxidation to quaternary salts.

6-Oxo derivative is N-substituted amide with terciary nitrogen that does not form any salts. Its reduction with zinc in the presence of acid or with lithium aluminium hydride leads to dihydro derivative. Dihydro derivative can, on the other hand, be oxidized with potassium ferricyanide to oxo derivative.

No similar disproportionation in the alkaline solution of sanguinarine was observed[94,117].

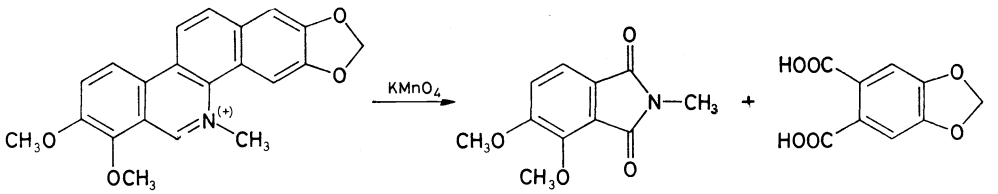
### *Oxidation*

Sanguinarine under treatment of potassium ferricyanide in alkaline medium[118] gives 6-oxosanguinarine (*87*) in the yield of 74%. Similarly chelirubine (*3*) was oxidized to 6-oxochelirubine (*88*) (ref.[119]).



| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $R^6$ |
|---|---|---|---|---|---|---|
| *84* | $OCH_2O$ | | H | OMe | OMe | H |
| *86* | $OCH_2O$ | | H | $OCH_2O$ | | H |
| *87* | $OCH_2O$ | | $OCH_2O$ | | H | H |
| *88* | $OCH_2O$ | | $OCH_2O$ | | H | OMe |

The oxidation with a stronger oxidation agent (potassium permanganate) leads to the decomposition of the skeleton used. During oxidation of chelerytrine (*2*) it was found that oxidation leads to the splitting of the bond between B and C ring under formation 4,5-methylenedioxyphthalic acid and N-methylamide of *o*-hemipinic acid (Scheme 7) (ref.[45]).



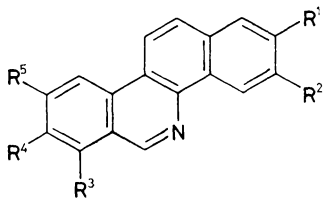

SCHEME 7

## 5.9. OTHER REACTIONS

Heating of sanguinarine (*1*) with zinc powder in the stream of hydrogen led to the elimination of the all substituents being at aromatic ring[42] forming benzo[*c*]phenanthridine (*89*).

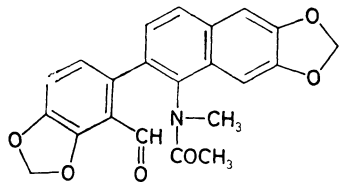
Sublimation of chelerythrine (*2*) at the temperature of 200°C gave norchelerythrine (*90*) (ref.[120]). Norchelerythrine (*90*) and norsanguinarine (*91*) were prepared[121] in the high yields of about 90% heating the corresponding quaternary salts on a bath at 205°C. Norsanguinarine (*91*) was also prepared by the heating of sanguinarine (*1*) with benzylalcohol[34].

Pyrolysis of fagaronine (*10*) at the temperature of 270°C led to the mixture of three products[122]: N-demethylfagaronine (*92*) in the yield of 89·5%, 2,3,8,9-tetramethoxybenzo[*c*]phenanthridine (*93*) (2%) and 2,8-dihydroxy-3,9-dimethoxybenzo[*c*]phenanthridine (*94*) (4%).



| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ |
|---|---|---|---|---|---|
| *89* | H | H | H | H | H |
| *90* | $OCH_2O$ | | OMe | OMe | H |
| *91* | $OCH_2O$ | | $OCH_2O$ | | H |
| *92* | OH | OMe | H | OMe | OMe |
| *93* | OMe | OMe | H | OMe | OMe |
| *94* | OH | OMe | H | OH | OMe |

The heating of sanguinarine pseudobase with acetanhydride led to the ring opening under formation of N-acetylsanguinarine (*95*) the only described derivative with aldehyde group[34]. But again no spectral data of its identification were given.



*95*

The reaction of fagaridine (*11*) with diazomethane led to chelerythrine (*2*). The reaction was carried out in the connection with the structural establishment of fagaridine from *Fagara xanthoxyloides*[55].

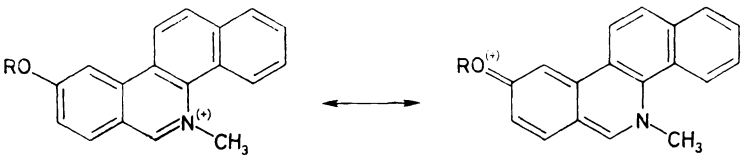
## 6. PHARMACOLOGY AND TOXICOLOGY

The principal pharmacological effects of the QBPAs studied at the present time are: antimicrobial, antifungal, antiinflammatory and antitumor activities.

The main effects on biological activity are influenced by: (i) the planarity of the aromatic molecule, (ii) acidity of quaternary cation (i.e. equilibrium of pseudobase formation) and (iii) the position of substituents on ring D. It seems that the nature of substituents on ring A has no significance on the type of the biological activity.

Caolo and Stermitz[26] explain that antitumor effects of nitidine (*7*) and fagaronine (*10*) are connected with the high stability of the iminium structure. The quaternary

cation is stabilized by resonance interaction of a non-bonded electron pair at C-9 alkoxy group with double iminium bond. This effect cannot be pronounced so well in case of C-7 substituted QBPAs (sanguinarine and chelerythrine types) due to steric hindrance of C-6 hydrogen (Scheme 8).




SCHEME 8

Nitidine chloride (7) exhibits high activity in P-388 mouse leukemia[69]. The bond $C{=}N^+$ and the substituents on ring D are considered to be the critical site of the molecule. Blocking this region by hindrance of the perigroup at C-7 (sanguinarine, chelerythrine) or removing the iminium bond (by formation of 6-oxo or 5,6-dihydro derivatives) leads to inactivation of the alkaloid.

The quaternary benzo[*c*]phenanthridine alkaloids of sanguinarine and chelerythrine type exhibit significant antimicrobial activity especially against gram positive bacteria[70,123,124].

This group of alkaloids exhibits also a very good antifungal activity upon some strains e.g. *Trichophyton*[129], *Microsporum*[130], *Candida*[123,124,129,130]. The combined therapy with QPBAs (local application) and griseofulvin (per os) was applied in experimental mycoses in guineapigs[130].

The antiinflammatory activity of sanguinarine and chelerythrine has been tested on experimental oedema in rats and has been found to be very notable. After subcutaneous application the results were comparable with indomethacin. Sanguinarine showed a higher activity than chelerythrine[123].

Since 1981 clinical trials of the preparation Sanchelin containing a mixture of sanguinarine and chelerythrine (isolated from *Chelidonium majus* L.) have been conducted at the Department of Stomatology, Palacký University, Olomouc (Czechoslovakia). The concentration of effective substances is 0·05%. As a vehicle the 4% carboxymethylcellulose gel (prepared extemporaneously) has been applied. The preparation is indicated for a local therapy of acute inflammations or acute exacerbation of chronic inflammations of gingiva[125–127].

The Soviet preparation Sangviritrin[10] is a purified, non-separated mixture of sanguinarine and chelerythrine hydrogensulfates in the ratio 1 : 1 to 1 : 2. It has been obtained from *Macleaya cordata* (WILLD.) BR. R. and *M. microcarpa* (MAXIM.) FEDDE as well as *Chelidonium majus* L. Since 1982 it has been used in three application forms: 1% liniment, 0·2% solution in 30% ethanol and tablets

(per 5 mg) to prepare aqueous solutions extemporaneously. There is a wide employing of Sangviritrin as an antimicrobial agent: 0·1% aqueous solution to rinse wounds in surgery, in various inflammatory affections in dental care (aphtae, stomatitis, parodontosis, gingivitis), ENT-applications (tonsilitis, pharyngitis, a state after tonsillectomy) – 0·02% oral rinse, further in gynecology and dermatology (liniment at dermatomycoses and microbial eczema)[10].

In western countries the fraction of quaternary benzo[*c*]phenanthridine alkaloids from *Sanguinaria canadensis* L. is utilized especially in dental applications. Sanguinaria extract is a component of some commercial toothpastes and oral rinses (e.g. Viadent, Vipont Laboratories, U.S.A.; Veadent, Potter and Clark Ltd., U.K.). At present the literature dealing with this subject is getting very rich and not providing an easy survey.

In 1984 sanguinarine was reported as a new anti-plaque agent. A rinsing with the solution containing 0·045% of sanguinaria extract reduced the plaque amounts by 19% during 8-day test period[81]. Another study demonstrated the effect of sanguinaria

TABLE VIII

Acute toxicity of sanguinarine (*1*) and chelerythrine (*2*) and their mixtures

| Alkaloid | $LD_{50}$ (mg/kg) | Application[a] | Animals | Ref. |
|---|---|---|---|---|
| Sanguinarine | 20[b] | i.p. | rats | 105 |
| Sanguinarine | 19·4 | i.v. | mice | 135 |
| Sanguinarine | 15·9 | i.v. | mice | 123 |
| Sanguinarine | 102[b] | s.c. | mice | 123 |
| Sanguinarine | 1 658 | p.o. | rats | 29 |
| Sanguinarine | 29 | i.v. | rats | 29 |
| Chelerythrine | 18·5 | i.v. | mice | 123 |
| Chelerythrine | 95[b] | s.c. | mice | 123 |
| Sangviritrin[c] | 14·2 | i.p. | mice | 10 |
| Sangviritrin[c] | 12 | i.p. | rats | 10 |
| Sangviritrin[c] | 470 | i.g. | mice | 10 |
| Sangviritrin[c] | 500 | i.g. | rats | 10 |
| Sanguinaria extract[d] | 200 | i.c. | rabbits | 29 |
| A mixture isolated from *Ch. majus*[e] | 510 | p.o. | mice | 125 |

[a] i.p. (intraperitoneal), i.v. (intravenous), s.c. (subcutaneous), i.g. (intragastric), i.c. (intracutaneous), p.o. (per os); [b] $LD_{100}$; [c] a purified mixture of sanguinarine and chelerythrine hydrogensulfates in the ratio 1 : 1 to 1 : 2 approximately; [d] for the composition see Table III, reference[29]; [e] chelerythrine and sanguinarine (7 : 3).

extract on controlling gingivitis[131]. However, there are studies not confirming the efficiency of sanguinaria agent against developing plaques or gingivitis[132–134].

The toxicity (acute and chronic) of sanguinarine type alkaloids was studied by many authors (Table VIII). According to recent results it seems they are relatively low toxic substances. It is a favourable matter for their pharmacological prospects.

*Chelidonium majus* L. represents the natural source of QBPAs in Central Europe. This species contains a considerable excess of chief alkaloid chelidonine (1·0–1·5%) which can be converted to pure sanguinarine[33,34]. Dicranostigma lactucoides HOOK.F. et THOMS. seems to be the most advantageous source of QBPAs. This species can be cultivated without major difficulties under Czechoslovak climatic conditions and it affords good yields of the root[128].

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