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Phytochemistry 68 (2007) 150–175

PHYTOCHEMISTRY

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Review

Quaternary protoberberine alkaloids

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Received 30 June 2006; received in revised form 18 September 2006
Available online 15 November 2006

Dedicated to Professor Jiří Slavík on the occasion of his 85th birthday.

Abstract

This contribution reviews some general aspects of the quaternary iminium protoberberine alkaloids. The alkaloids represent a very extensive group of secondary metabolites with diverse structures, distribution in nature, and biological effects. The quaternary protoberberine alkaloids (QPA), derived from the 5,6-dihydrodibenzo[*a*,*g*]quinolizinium system, belong to a large class of isoquinoline alkaloids.

Following a general introduction, the plant sources of QPA, their biosynthesis, and procedures for their isolation are discussed. Analytical methods and spectral data are summarized with emphasis on NMR spectroscopy. The reactivity of QPA is characterized by the sensitivity of the iminium bond $C{=}N^+$ to nucleophilic attack. The addition of various nucleophiles to the protoberberine skeleton is discussed. An extended discussion of the principal chemical reactivity is included since this governs interactions with biological targets.

Quaternary protoberberine alkaloids and some related compounds exhibit considerable biological activities. Recently reported structural studies indicate that the QPA interact with nucleic acids predominantly as intercalators or minor groove binders. Currently, investigations in many laboratories worldwide are focused on the antibacterial and antimalarial activity, cytotoxicity, and potential genotoxicity of QPA.

Keywords: Berberidaceae; Quaternary protoberberine alkaloid; Plant source; Nucleophilic addition; NMR spectroscopy; X-ray diffraction; Cytotoxicity; DNA binding

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Contents

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doi:10.1016/j.phytochem.2006.10.004

1. Introduction

1.1. General aspects

The alkaloids represent a very extensive group of secondary metabolites, with diverse structures, distribution in nature, and important biological activities. A simple general definition (Roberts and Wink, 1998) of an alkaloid was first suggested by Pelletier in 1983: “An alkaloid is a cyclic compound containing nitrogen in a negative oxidation state which is of limited distribution in living organisms”. This definition includes both alkaloids with nitrogen as part of a heterocyclic system and many exceptions with exocyclic nitrogen, such as colchicines or capsaicin.

Generally, the function of secondary metabolites in plants is not unequivocally determined. Concerning the alkaloids, their protective mechanism against herbivore animals and possibly some parasites is assumed. In contrast, these plants can become the targets for insects that specialize in the alkaloid-producing plants (e.g., bark tree). However, strong physiological effects and the selectivity of some alkaloids present opportunities for utilizing the alkaloids in human medicine.

The isoquinolines are one of the largest groups of alkaloids. The isoquinoline skeleton is a basic building block of various types of alkaloids including benzylisoquinolines, protopines, benzo[*c*]phenanthridines, protoberberines, and many others. The protoberberine alkaloids are biogenetically derived from tyrosine. The quaternary protoberberine alkaloids (QPA) represent approximately 25% of all the currently known alkaloids with a protoberberine skeleton isolated from natural sources. This article is focused mainly on the iminium QPA because of their interesting chemical reactivity and biological activities.

Berberine is probably the most widely distributed alkaloid of all. In 1824, Hüttenschmid found a yellow coloring matter in what he believed to be *Geoffroya inermis*, the Jamaica cabbage-tree, and gave it the name jamaicine. In

Table 1
2,3,9,10-Tetrasubstituted QPAs

Alkaloid	2	3	9	10
Berberine	O–CH$_2$–O		OCH$_3$	OCH$_3$
Coptisine	O–CH$_2$–O		O–CH$_2$–O	
Palmatine	OCH$_3$	OCH$_3$	OCH$_3$	OCH$_3$
Jatrorrhizine	OCH$_3$	OH	OCH$_3$	OCH$_3$
Columbamine	OH	OCH$_3$	OCH$_3$	OCH$_3$
Thalifendine	O–CH$_2$–O		OCH$_3$	OH
Stepharanine	OH	OCH$_3$	OCH$_3$	OH
Groendlandicine	OCH$_3$	OH	O–CH$_2$–O	
Dehydrocorydalmine	OCH$_3$	OCH$_3$	OCH$_3$	OH
Dehydrodiscretamine	OCH$_3$	OH	OCH$_3$	OH
Dehydrocheilanthifoline	OH	OCH$_3$	O–CH$_2$–O	
Demethylenberberine	OH	OH	OCH$_3$	OCH$_3$
Epiberberine (Dehydrosinactine)[a]	OCH$_3$	OCH$_3$	O–CH$_2$–O	

[a] Synonym is given in the parentheses.

1826, Chevallier and Pelletan found a rich yellow alkaloid in the bark of *Xanthoxylum clava herculis*, and named it xanthopicrite. Both of these substances were subsequently proved to be identical with berberine, obtained by Buchner and Herberger as a yellow extract from *Berberis vulgaris* in 1830 (Felter and Lloyd, 1898).

Other representatives of the QPA, including the well-known alkaloids palmatine, jatrorrhizine, columbamine, and coptisine, are summarized in Tables 1–4.

Table 2
2,3,10,11-Tetrasubstituted QPAs

Alkaloid	2	3	10	11
Pseudocoptisine	O–CH_2–O		O–CH_2–O	
Pseudopalmatine	OCH_3	OCH_3	OCH_3	OCH_3
Pseudojatrorrhizine	OCH_3	OH	OCH_3	OCH_3
Pseudoepiberberine	OCH_3	OCH_3	O–CH_2–O	
Pseudocolumbamine	OH	OCH_3	OCH_3	OCH_3
Dehydrodiscretine	OCH_3	OH	OCH_3	OCH_3
Dehydrocoreximine	OH	OCH_3	OCH_3	OH
Thalifaurine	OCH_3	OH	O–CH_2–O	

1.2. Protoberberine skeleton

Most protoberberine alkaloids exist in plants either as tetrahydroprotoberberines or as quaternary protoberberine salts, although a few examples of dihydroprotoberberines have also been described. 5,6-Dihydrodibenzo[*a*,*g*]quinolizinium ($C_{17}H_{14}N^+$) is the basic skeleton of the quaternary protoberberine alkaloids (Scheme 1).

Substituents are usually present at positions 2,3,9,10 or 2,3,10,11 and the prefix pseudo- is often used for the latter substitution pattern. For example, pseudopalmatine, 2,3,10,11-tetramethoxy-5,6-dihydrodibenzo[*a*,*g*]quinolizinium, is a regioisomer of palmatine, 2,3,9,10-tetramethoxy-5,6-dihydrodibenzo[*a*,*g*]quinolizinium. 2,3,9,10-Tetrasubstituted and 2,3,10,11-tetrasubstituted QPA are summarized in Tables 1 and 2, respectively.

Additionally, compounds with substituents at carbon atoms C-1, C-4, C-5 and C-13 have been isolated from natural sources. The typical substituents are hydroxy groups, methoxy groups, and O–CH_2–O groups. The prefix retro- is used for protoberberines characterized by the

Table 3
1,2,*x*,*y*-Tetrasubstituted, pentasubstituted, and hexasubstituted QPAs

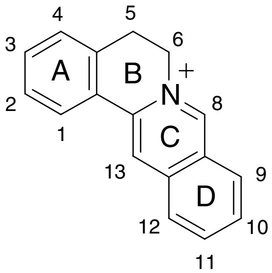
Alkaloid	1	2	3	4	5	8	9	10	11	12	13
Corysamine (Worenine)[a]		O–CH_2–O					O–CH_2–O				CH_3
Dehydrothalictrifoline		OCH_3	OCH_3				O–CH_2–O				CH_3
Dehydrothalictricavine		O–CH_2–O					OCH_3	OCH_3			CH_3
Dehydrocorydaline		OCH_3	OCH_3				OCH_3	OCH_3			OCH_3
Dehydroapocavidine		OH	OCH_3				O–CH_2–O				CH_3
Berberastine		O–CH_2–O			OH		OCH_3	OCH_3			
Thalidastine		O–CH_2–O			OH		OCH_3	OH			
Fissisaine		OCH_3	OCH_3	OH			OCH_3	OH			
Lincagenine		OCH_3	OCH_3	OH			OCH_3	OCH_3			
Stephabine	OH	OCH_3	OCH_3					OCH_3	OCH_3		
Dehydrocapaurimine	OH	OCH_3	OCH_3				OCH_3	OH			
13-Me-Pseudoepiberberine		OCH_3	OCH_3					O–CH_2–O			CH_3
PO-4 (Dehydroorientalidine)[a]	OCH_3	O–CH_2–O						OCH_3	O–CH_2–O–CH_2		
PO-5 (Alborine)	OCH_3	O–CH_2–O						OCH_3	OCH_3	CH_2OH	
Mequinine	OCH_3	OCH_3	OH			OCH_3					
Caseadine	OH	OCH_3						OCH_3	OCH_3		

[a] Synonym is given in the parentheses.

Table 4
Synthetic protoberberines and isolation artifacts

Alkaloid	2	3	4	8	9	10	11	13	Ref.
Berberrubine (Chileninone)[a]	O–CH_2–O				OH	OCH_3			Shamma and Rahimizadech (1986)
Palmatrubine	OCH_3	OCH_3			OH	OCH_3			Shamma et al. (1977) and Siwon et al. (1981)
Dehydroscoulerine	OH	OCH_3			OH	OCH_3			Schneider and Zenk (1993b) and Bjorklund et al. (1995)
MDD-coralyne		O–CH_2–O		CH_3		OCH_3	OCH_3		Makhey et al. (2000)
DM/II/33		O–CH_2–O				OCH_3	OCH_3		Goldman et al. (1997)
5,6-Dihydrocoralyne	OCH_3	OCH_3		OCH_3		OCH_3	OCH_3		Wang et al. (1996)
Glabrine	OCH_3	OCH_3			OCH_3	OH	OH		Bhakuni and Jain (1986)
Glabrinine	OCH_3	OCH_3			OCH_3	OCH_3	OCH_3		Bhakuni and Jain (1986)
Dehydrocorybulbine	OCH_3	OH			OCH_3	OCH_3		CH_3	Šantavý (1979)
13-Aminopalmatine	OCH_3	OCH_3			OCH_3	OCH_3		NH_2	McCalla et al. (1994)

[a] Synonym is given in the parentheses.



Scheme 1.

presence of an extra substituent as a side chain of ring D – position C-12 (PO-4, PO-5, mecambridine, and orientalidine fall among the retroprotoberberines). In the past, the prefix epi- was used for interchanged 2,3 and 9,10 substitution patterns (see, for example: berberine vs. epiberberine in Table 1). Various 1,2,*x*,*y*-tetrasubstituted, pentasubstituted, and hexasubstituted QPA are summarized in Table 3.

Two compounds were recently published as new quaternary protoberberine alkaloids. However, their structures are identical with alkaloids that have been known for a long time. Burasaine (Kluza et al., 2003) is identical with palmatine and pangrenine (Siddikov et al., 2005) is identical with alborine (PO-5). Bisjatrorrhizine, formed by an ortho-oxidative coupling of the phenolic group of jatrorrhizine (Fajardo et al., 1996), should also be included in this review. Table 4 contains a small part of the quaternary protoberberine alkaloids which are not natural, but they were synthesized from their natural precursors or formed artificially during the isolation processes. Although palmatrubine has been described as a natural product (Shamma et al., 1977; Siwon et al., 1981), it is assumed to originate from the rearrangement of palmatine during the isolation process (cf. berberrubine (Shamma and Rahimizadech, 1986)).

In addition to the semi-synthetic protoberberine alkaloids summarized in Table 4, coralyne, 8-methoxy-5,6-dehydropseudopalmatine with a fully aromatic skeleton has been described (Wang et al., 1996; Goldman et al., 1997; Makhey et al., 2000). The other aromatic compounds are deoxythalidastine and dehydroberberrubine (Shamma and Dudock, 1965). Deoxythalidastine (dehydrothalifendine) is probably an artifact formed by dehydration of the known 5-hydroxyprotoberberine alkaloid thalidastine (Ikuta and Itokawa, 1982). The structures of compounds with a completely aromatic core are shown in Scheme 2.

2. Plant sources

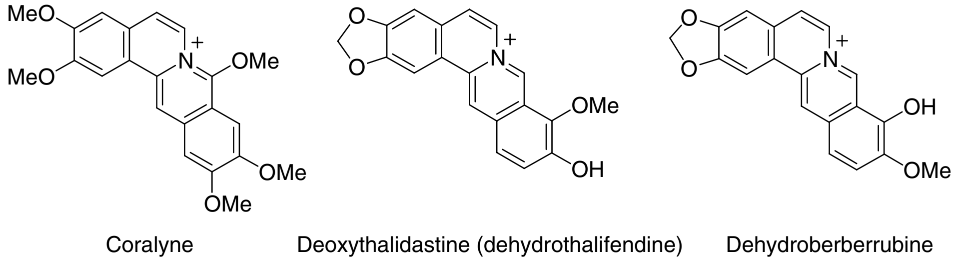
The protoberberines are distributed in such plant families as Papaveraceae, Berberidaceae, Fumariaceae, Menispermaceae, Ranunculaceae, Rutaceae, Annonaceae, as well as a few examples in Magnoliaceae and Convolvulaceae (Bentley, 1997, 1998a,b, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006). The occurrence of a few representatives of the QPA is restricted to only the one plant species. The distributions of QPA in plant families and individual species are summarized in Table 5.

The protoberberine alkaloids are rather widely distributed in at least six plant families. There are more than one hundred of these compounds (tetrahydroprotoberberines, quaternary protoberberines) (Bentley, 1997, 1998a,b, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006). However, the number of known quaternary iminium alkaloids is significantly smaller (see Tables 1–3). In plant tissues, the positive charge of the QPA is compensated for by chloride anions or the anions of organic acids, such as succinic acid (Pervushkin et al., 1999). Generally, anions are exchanged during the isolation process and the QPA are frequently obtained in the form of chlorides, iodides, or perchlorates.

Quantitative and qualitative differences in the alkaloid content of the plant tissues are affected by several factors, e.g., climate, environment, and soil composition. The content of the individual alkaloids is also somewhat influenced by the vegetative season (Tomé and Colombo, 1995). The occurrence of several alkaloids is restricted to a specific part of the plant body, whereas other compounds are distributed in the whole body with varying rates in different tissues.

3. Biosynthesis

It has been shown (investigation of *Hydrastis canadensis*) (Gear and Spenser, 1963) that tyrosine is a very efficient precursor of berberine and it is incorporated into both the



Scheme 2.

Table 5
Selected plant sources of QPAs

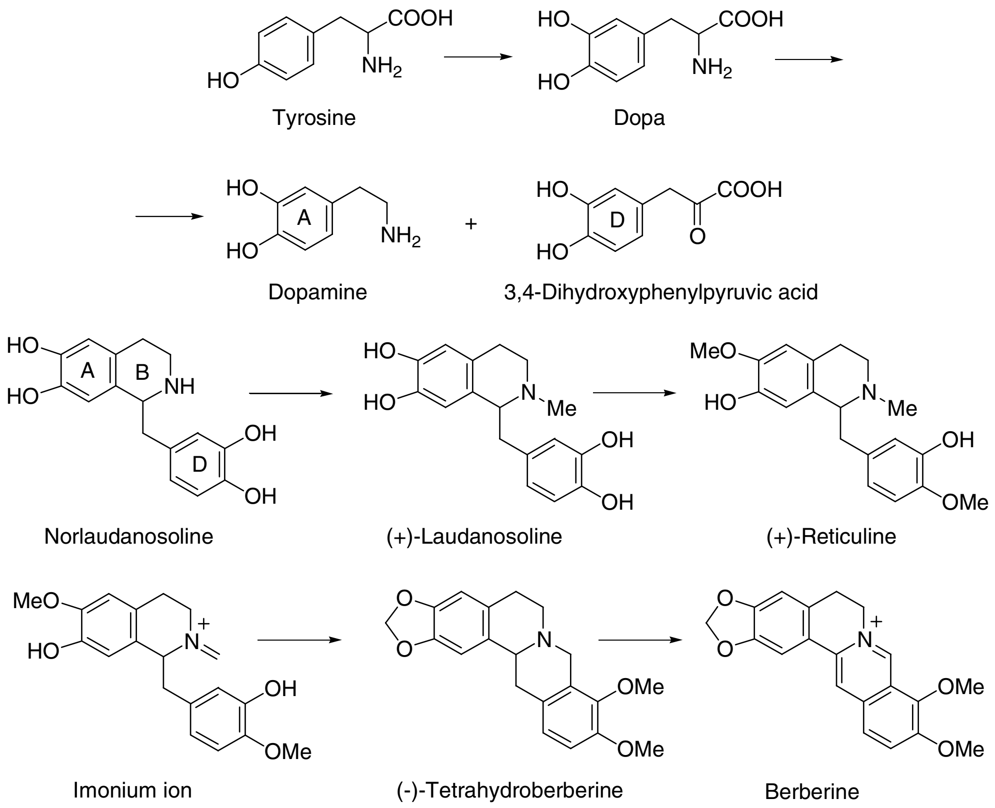
Alkaloid	Typical plant species/family	Refs.
Berberine	Berberidaceae and others	1, 2, 3, 4, 33, 34, 39, 43, 44, 52, 53
Coptisine	Fumariaceae, Papaveraceae	2, 4, 48, 49, 52
Palmatine	Berberidaceae and others	2, 3, 4, 5, 37, 38, 39, 41, 42, 51, 54
Jatrorrhizine	Berberidaceae and others	1, 2, 3, 5, 33, 37, 39, 41, 50, 51, 54
Columbamine	*Berberis vulgaris*, *Enantia chlorantha*	3, 6, 7, 37, 38, 39, 45, 51
Thalifendine	*Thalictrum sp.*	1, 3, 6, 27, 30, 33, 37
Stepharanine	*Xylopia parviflora*, *Stephania glabra*	5, 8, 9
Groendlandicine	*Coptis groendlandicum*	10
Dehydrocorydalmine	*Stephania glabra*	9, 11
Palmatrubine	*Thalictrum polygamum*	12, 13
Dehydrodiscretamine	*Fissistigma balansae*, *Thalictrum foliolosum*	3, 7
Dehydrocheilanthifoline	*Berberis cordata*	10
Demethylenberberine	*Thalictrum javanicum*	1, 14, 32, 33
Epiberberine (Dehydrosinactine)[a]	*Coptis chinensis*, *D. floribunda (aristata)*[a]	15, 35, 36
Pseudocoptisine	*Isopyrum thalictroides*	2, 16
Pseudopalmatine	*Stephania suberosa*, *Penianthus zenkeri*	2, 21, 26, 34
Pseudojatrorrhizine	*Fibraurea chloroleuca*	13
Pseudoepiberberine	–[b]	2, 6
Pseudocolumbamine	*Enanthia chlorantha*, *Fibraurea chloroleuca*	13, 17
Dehydrodiscretine	*Heptacylum zenkeri*, *Thalictrum fauriei*	5, 27, 28
Dehydrocoreximine	*Xylopia pauriflora*	5
Thalifaurine	*Thalictrum fauriei*	1
Caseadine	*Ceratocapnos heterocarpa*	29
Corysamine (Worenine)[a]	*Chelidonium majus*	1, 2
Dehydrothalictrifoline	–[b]	6
Dehydrothalictricavine	–[b]	6
Dehydrocorydaline	*Corydalis bulbosa*	18
Dehydroapocavidine	*Corydalis cava*	1
Berberastine	*Hydrastis canadensis*	6, 19, 40, 46
Thalidastine	*Thalictrum sp.*	3, 6, 30, 31
Fissisaine	*Fissistigma balansae*	7
Lincagenine	*Guatteria schomburgiana*, *G. sessilis*	20
Stephabine	*Stephania suberosa*	21
Dehydrocapaurimine	*Coptis platicarpa*	10
13-Methyl pseudoepiberberine	–[b]	6
PO-4	*Papaver orientale*	10, 22, 23, 24, 47
Alborine (PO-5)[a]	*Papaver orientale*	1, 10, 22, 23, 24, 47
Mequinine	*Meconopsis quintuplinerva*	25

1. Bhakuni and Jain (1986), 2. Pavelka and Smékal (1976), 3. Chattopadhyay et al. (1983), 4. Ro et al. (2001), 5. Nishiysma et al. (2004), 6. Shamma et al. (1969), 7. Chia et al. (1998), 8. Cava et al. (1968), 9. Doskotch et al. (1967), 10. Šantavý (1979), 11. Kaneko and Naruto (1969), 12. Shamma et al. (1977), 13. Siwon et al. (1981), 14. Bahadur and Shukla (1983), 15. Yang et al. (1998), 16. Moulis et al. (1977), 17. Bourdat-Deschamps et al. (2004), 18. Miyazawa et al. (1998), 19. Jeffs (1967), 20. Orfila et al. (2000), 21. Patra et al. (1987), 22. Shamma (1972), 23. Preininger et al. (1970), 24. Šimánek et al. (1970), 25. Bentley (1997), 26. Tane et al. (1997), 27. Wright et al. (2000), 28. Duah et al. (1983), 29. Suau et al. (1993), 30. Ikuta and Itokawa (1982), 31. Gao et al. (1987), 32. Cava and Reed (1967), 33. Pan et al. (2002), 34. Suau et al. (1991), 35. Bauer and Zenk (1991), 36. Drost et al. (1974), 37. Lopez et al. (1988), 38. Bonora et al. (1990), 39. Hsieh et al. (2004), 40. Weber et al. (2003), 41. Suau et al. (1998), 42. Nishiysma et al. (2004), 43. Košt'álová et al. (1982), 44. Valencia et al. (1984), 45. Svendsen et al. (1984), 46. Iwasa et al. (1982), 47. Preininger (1986), 48. Henry (1949), 49. Murugesan and Shamma (1980), 50. Lloyd (1898), 51. Rueffer and Zenk (1986), 52. Barreto et al. (2003), 53. Kumar et al. (2003), 54. Slavík and Slavíková (1995).

[a] Synonym is given in the parentheses.

[b] Source not described in the reference.

top (ring A and part of ring B) and the bottom (ring D and part of ring C) parts of the alkaloid (Gear and Spenser, 1963). However, if labeled dopamine is fed in, only one molecule of this species is incorporated into the alkaloid. Tyrosine must, therefore, give rise to two different intermediates during the biosynthetic process, one of them being dopamine. Although not all of the individual steps have yet been completely established, the general sequence shown in Scheme 3 seems to prevail in the biosynthesis of berberine (Shamma, 1972; Frick and Kutchan, 1999; Hara et al., 1994). Several enzymes (Beecher and Kelleher, 1984; Kobayashi et al., 1987; Amann et al., 1988; Jendrzejewski, 1990; Schneider and Zenk, 1993a) participate in the transformation pathway from reticuline to berberine (Kametani et al., 1976; Jeffs and Scharver, 1976; Bhakuni et al., 1983), one of them is berberine bridge enzyme (Frenzel et al., 1988; Rueffer and Zenk, 1994; Kutchan and Ditrich, 1995). Tetrahydroprotoberberine oxidase is employed in the conversion of tetrahydroprotoberberine to the quaternary form (Amann et al., 1984, 1988). Subsequently, the



Scheme 3.

berberine can be converted into other protoberberines, e.g., jatrorrhizine (Rueffer et al., 1983).

4. Isolation and separation

The whole plant or just a selected part such as the bark or the stem is used for isolating the principal constituents or the minor components. Typically (Táborská et al., 1994), the part of the plant with the highest alkaloid content is dried at room temperature. The extraction of alkaloids from the pulverized or finely cut dried plant material is based on maceration and percolation, or is performed using a Soxhlet apparatus. The dried plant tissue is extracted for a long time (days) using methanol, with the end of the extraction indicated (Košt'álová et al., 1982) by Dragendorff's reagent. Traditional methods such as crystallization, column chromatography, and extraction are frequently used to obtain the individual alkaloids from the plant extract. Afterwards highly sensitive and sophisticated methods for separating (various chromatographic methods including HPLC) and identifying (spectroscopic methods) the constituents are used (see below).

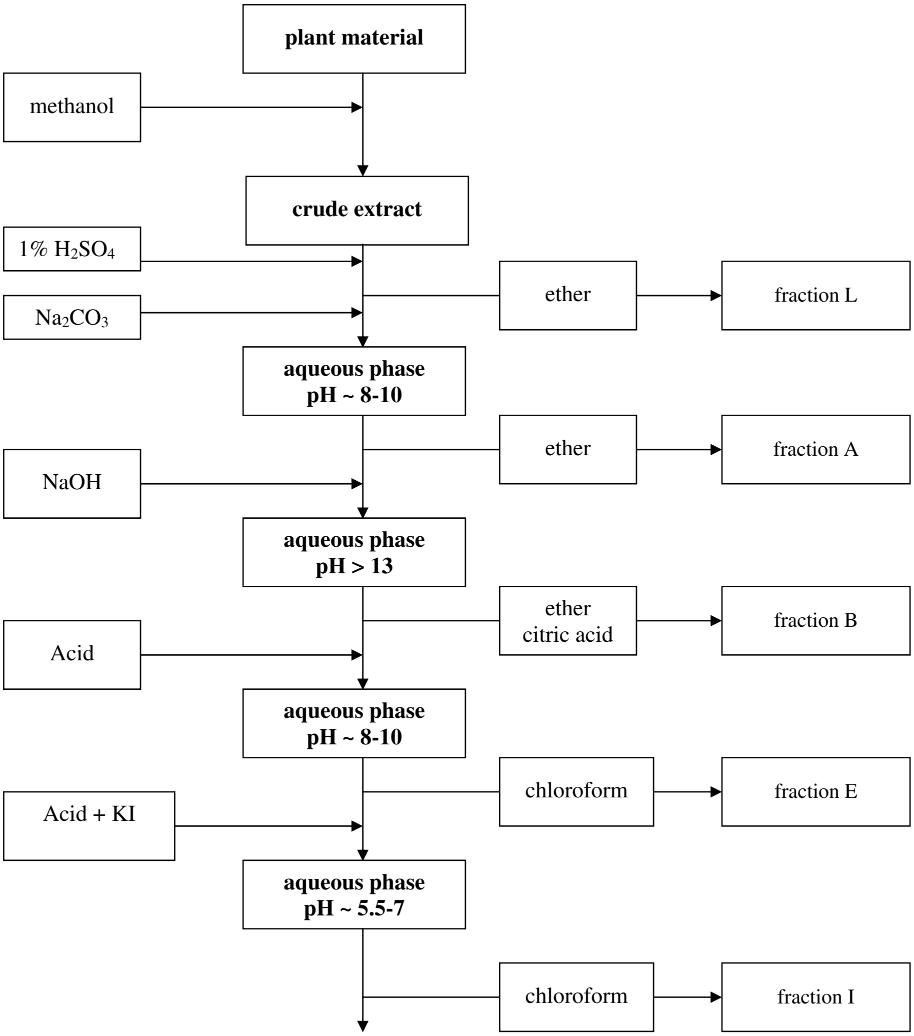
Separation of the QPA from the crude plant extract containing various kinds of alkaloids can be performed using several methods. A general isolation procedure was reported by Slavíková and Slavík (1966). The individual steps of the published sequence are shown in Scheme 4.

The isolation procedure is based on the interconversion reaction between the protoberberine salt (soluble in water, stable in acidic and neutral media) and the base (soluble in organic solvents). During the isolation and purification process the protoberberine salts are converted into their corresponding bases (Marek et al., 2003b) and then extracted into an organic solvent. This principal reactivity is discussed in further detail in Chapter 6.

The individual components were subsequently separated from fraction B by using chromatographic methods and recrystalization. Column chromatography of palmatine chloride, berberine chloride, columbamine chloride, and jatrorrhizine chloride on acid-washed alumina was used to separate the QPA from the crude extract.

Chloroform with increasing content of methanol was used as the mobile phase (Cava and Reed, 1965). The paper chromatography R_F values of the alkaloid chlorides for different solvent systems (Cava and Reed, 1965) are summarized in Table 6.

In addition to the standard chromatography, several modern chromatographic techniques have been used for separating and identifying the QPA. TLC analysis of QPA can present some problems because these compounds are highly polar and standard eluents are not suitable for their separation. Overpressure layer chromatography (OPLC) is one of the methods which resolve these complications (Pothier et al., 1993). OPLC on silica gel plates with ternary eluents was applied to the complete separation of



Fraction L	lipophilic compounds and non-basic alkaloids
Fraction A	quaternary benzophenantridine alkaloids, protopine alkaloids, tertiary bases soluble in ether
Fraction B	citrates of quaternary protoberberine alkaloids
Fraction E	the rest of fraction A, non-polar compounds
Fraction I	highly polar quaternary alkaloids (as iodides)

Scheme 4.

quaternary salts. This method can be used to separate the alkaloids from plant extracts and to determine the hR_F values of some authentic standards. Reversed-phase HPLC analysis (Táborská et al., 1994) of the extract from *Chelidonium majus* was used to separate 27 alkaloids from a mixture of 28 compounds. However, with HPLC many peaks cannot be assigned to the individual species (the same problem occurs with TLC). This complication can be resolved by using ion-pair HPLC and utilizing sodium perchlorate for the extraction (Kato et al., 1996).

One of the problems connected with the isolation procedures for the QPA on chromatographic columns is a possible structural rearrangement of the natural QPA leading to unnatural rearranged compounds and isolation artifacts (see Chapter 6.1). Some isolation artifacts (Marek et al., 2003b) were classified as natural QPA for a long time. For example, berberrubine was described as a natural product (Shamma et al., 1977; Peteu, 1965), but since 1986 it has been known to be an isolation artifact (Shamma and Rahimizadech, 1986).

5. Identification and analytical methods

Currently, standard spectroscopic methods for investigating the structure of natural products comprise nuclear

Table 6
R_F values of QPA chlorides in several solvent mixtures (paper chromatography)

System	Berberine	Palmatine	Jatrorrhizine	Columbamine
A	0.38	0.35	0.27	0.24
B	0.77	0.75	0.74	0.73
C	0.83	0.83	0.69	0.60
D	0.68	0.66	0.73	0.69
E	0.53	0.56	0.37	0.29
F	0.53	0.53	0.38	0.37

(A) 100 ml butanol, 100 ml chloroform, 100 ml water; (B) 160 ml butanol, 20 ml glacial acetic acid, 100 ml water; (C) 50 ml pentan-2-ol, 50 ml isoamyl alcohol, 140 ml 28% formic acid, 36 ml chloroform; (D) 92 ml pyridine, 225 ml ethyl acetate, 200 ml water; (E) 200 ml butanol, 100 ml 7% (aq.) ammonia; (F) 160 ml propanol, 40 ml 7% (aq.) ammonia.

magnetic resonance (NMR), infrared spectroscopy (IR), and ultraviolet spectroscopy (UV), and these are often combined with mass spectrometry (MS). Single-crystal X-ray diffraction is a powerful technique used for determining the molecular topology. However, this technique suffers from the necessity of obtaining high-quality single crystals. Applications of the above mentioned methods to the structural analysis and characterization of the QPA are discussed in the following subchapters.

The quaternary protoberberine alkaloids are characterized by bright colors. Their colors range from yellow (berberine) to orange (jatrorrhizine). The color of the crystalline salts may vary depending on the method of crystallization or purification or both. The colors of their solutions may also vary slightly, depending on the concentration, solvent, and pH. The melting points of the QPA salts usually range from 200 to 300 °C.

5.1. Mass spectrometry

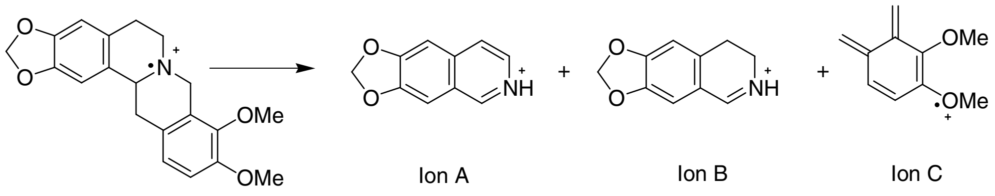
Mass spectrometry has been a powerful tool for investigating the structures of complex molecules and natural products for a long time. It is frequently used for characterizing the QPA in crude mixtures of alkaloids or in plant extracts. The base peaks produced by ESI-MS without CID (collision induced dissociation) of the abundantly occuring QPA are: berberine 336, palmatine 352, jatrorrhizine 338, columbamine 338, coptisine 320, epiberberine 336, and berberastine 322. The ESI-MS spectra with CID (−30 V) contain 4–5 fragments (Chuang et al., 1996). Fragmentations of the quaternary alkaloids berberine, jatrorrhizine, coptisine, palmatine, and 13-methylberberine using electrospray ionization tandem mass spectrometry (ESI-MS) have been described recently (Wang et al., 2004a,b). For example, the fragmentation pathway of berberine ($M_r \rightarrow \rightarrow MS^5$) has been described by the following sequence: 336 (*m*/*z*, berberine) → 321 ($-CH_3^{\bullet}$) → 320 ($-H^{\bullet}$) → 318 (–2H) → 290. The ESI-MS technique has been used for the structural characterization and identification of the alkaloids in Rhizoma Coptidis (for details, see Wang et al., 2004a,b).

9-Hydroxy-10-methoxy compounds can be differentiated from their 9-methoxy-10-hydroxy regioisomers (Shamma, 1972). Those with the former substitution pattern preferentially eliminate a methyl group from the equivalent of ion C (Scheme 5), yielding an ion at 135, whereas the latter lose a hydrogen atom from their ion C equivalent, giving rise to a new ion at 149. Additionally, by careful analysis of peak intensities, it may even be possible to differentiate between the C-9, C-10 and C-10, C-11 substitution patterns (Habermehl et al., 1970; Schneider and Zenk, 1993a).

The mass spectrum of berberine base shows a very minor signal for the molecular ion due to the easy thermal dissociation of the C8–OH bond upon the formation of the quaternary alkaloid and to the disproportionation of berberine base into dihydroberberine and oxoberberine (Shamma, 1972; Dostál et al., 2004). Generally, the ion corresponding to the quaternary alkaloid dominates the spectrum of C8–X substituted dihydroprotoberberine, formed by the nucleophilic addition of X^- to the carbon C-8 (Dostál et al., 2004). The formation of the quaternary ion is due to the lability of the C8–X chemical bond and the thermal stability of the quaternary form.

5.2. UV–VIS spectroscopy

The pattern of the UV absorption spectra of the QPA bases is determined by the auxochromic groups bound to ring D (Pavelka and Smékal, 1976), the methoxy groups on carbons C-9 and C-10 or C-10 and C-11. Quaternary salts of protoberberine alkaloids in polar media exhibit four well-defined absorption bands. The ultraviolet absorption spectra of the pseudoberberine alkaloids are markedly different from those of the protoberberine alkaloids (Shamma et al., 1969). The only difference between the pseudoberberines and the berberines is the position of the substituents on aromatic ring D; in pseudoberberines the second auxochromic group is bound to carbon C-11,



Scheme 5.

in berberines to C-9 (see Chapter 1.2). The protoberberine salts show (Shamma et al., 1969) a minimum at 301–310 nm, while the pseudoberberine salts show strong absorption in this region in the form of a peak or shoulder. According to one of the research groups (Preininger et al., 1970), it is the oxygenous electron-donating substituent on C-11 that has the decisive effect on the polarization of a pseudoberberine molecule in the excited state.

The transition from the tetrahydroisoquinoline tertiary nitrogen in tetrahydroprotoberberine alkaloids to the quaternary nitrogen in the salts of protoberberine and pseudoprotoberberine bases manifests itself in the fluorescence spectra of these compounds by a characteristic bathochromic shift of the emission bands and by a considerable increase in the intensity of fluorescence.

The oxygenous electron-donating groups (methoxy and hydroxy) in the quaternary protoberberine alkaloids appreciably reduce the energy of electron transfers between the ground and the excited singlet state for these molecules, and, moreover, influence their absorbances and fluorescence intensities. The significant bathochromic shifts of the absorption and the fluorescence bands upon the introduction of these auxochromic groups into a protoberberine or tetrahydroprotoberberine molecule originate in the prolonged conjugated system of these molecules, as a result of the interaction of the free electron pairs of the oxygen with the conjugated π-electron system of the aromatic rings (Pavelka and Smékal, 1976). The values of λ_{max} of the QPA chlorides measured in ethanol are summarized in Table 7. However, the λ_{max} values of a few QPA with other anions measured in various solvents have also been published (Brezová et al., 2004).

Table 7
λ_{max} of QPA chlorides in ethanol (Pavelka and Smékal, 1976; Chia et al., 1998)

Alkaloid	λ_{max} (nm)
Berberine	230, 267, 344, 352, 432
Coptisine	229, 241, 268, 354, 363, 467
Palmatine	228, 240, 268, 276, 343, 350, 433
Berberrubine	234, 275, 353–8, 455
Jatrorrhizine	228, 241, 267, 352, 440
Pseudocoptisine	220, 231, 266, 289, 317, 346, 380
Pseudopalmatine	242, 265, 288, 310, 342, 380
Pseudoepiberberine	220, 240, 264, 290, 310, 341, 380
Columbamine[a]	206, 225, 265, 345
Corysamine	230, 240, 268, 344, 352, 455
Fissiaine	206, 230, 282, 337

[a] In methanol.

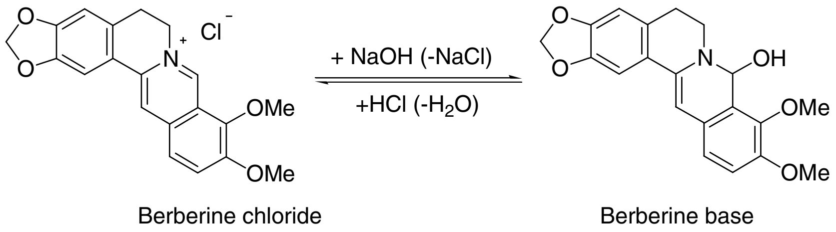
5.3. NMR spectroscopy

Nuclear magnetic resonance spectroscopy is the most powerful method used for investigating the structure of natural products in the solution (Martin and Crouch, 1994; Reynolds and Enriquez, 2002). Modern 1D and 2D NMR spectroscopy are capable of providing unequivocal information about the chemical bonding, i.e. the constitution of the molecule. However, the magnitudes of the indirect spin-spin coupling constants are used to describe the configurational and conformational aspects. Nowadays, the widespread application of the Karplus equation in determining the torsion angles represents a textbook example of the utilization of NMR to reconstruct 3D structures of molecules (Marquez et al., 2001). The determination of the Nuclear Overhauser Effect (NOE) between two nuclei (predominantly ^{1}H–^{1}H) is a complementary approach used to elucidate the molecular topology (Neuhaus and Williamson, 1989). Currently, ^{1}H–^{1}H homonuclear (COSY, DQF-COSY) and ^{1}H–^{13}C heteronuclear (HMQC, HSQC, HMBC) correlation techniques are routinely applied in the field of constitutional analysis. 1D or 2D NOE experiments are used predominantly for solving stereochemical problems.

The NMR chemical shift contains very detailed information about the electron distribution around the atomic nucleus. Structural modifications are strongly reflected in changes in the chemical shift. The influence of the interconversion between the salt and the base of the alkaloid berberine (Scheme 6) on the ^{1}H and ^{13}C chemical shifts is demonstrated in Table 8 (Blaskó et al., 1988; Jansen et al., 1989; Marek et al., 2003b).

Substantial changes in the chemical shieldings of H-8 ($\Delta\delta \sim 3.7$ ppm) and especially of C-8 ($\Delta\delta \sim 66$ ppm), the atom at the direct center of the structural change, are noticeable. However, the differences in the shieldings of the C-13 ($\Delta\delta \sim 25$ ppm) and N-7 ($\Delta\delta \sim 106$ ppm) atoms, which are components of the attacked heterocycle (ring C), reflect changes in the electronic structure to a large extent. The ^{1}H and ^{13}C NMR chemical shifts of several protoberberines are summarized in Tables 9 and 10.

During the past few years, ^{15}N NMR chemical shifts and ^{1}H–^{15}N coupling constants have become entrenched



Scheme 6.

Table 8
^{1}H and ^{13}C NMR chemical shifts (δ in ppm) of berberine chloride (DMSO-d_6) and berberine base (CD_2Cl_2) (Blaskó et al., 1988; Marek et al., 2003b)

Atom	δ_H		δ_C	
	Berberine chloride	Berberine base	Berberine chloride	Berberine base
1	7.79	7.15	105.43	104.96
2	–	–	147.63	147.29
3	–	–	149.76	147.82
4	7.09	6.62	108.43	108.16
4a	–	–	130.64	129.73
5	3.22	2.86/2.89	26.35	30.48
6	4.95	3.51/3.72	55.19	46.66
8	9.91	6.18	145.42	79.50
8a	–	–	121.37	123.46
9	–	–	143.61	146.17
10	–	–	150.36	150.33
11	8.20	6.94	126.67	114.58
12	8.01	6.90	123.53	119.72
12a	–	–	132.94	127.68
13	8.96	6.08	120.18	95.34
13a	–	–	137.41	136.72
13b	–	–	120.42	126.21
OCH_2O	6.17	5.93/5.95	102.07	100.99
9-OMe	4.10	3.91	61.95	61.01
10-OMe	4.07	3.85	57.06	56.07

as important restraints used for elucidating the structures of natural products (Martin and Hadden, 2000; Martin and Williams, 2005). Owing to the development of new NMR techniques and improvements in the spectrometer hardware, the detection of ^{15}N NMR spectra at natural abundance has become a routine task (Marek and Lyčka, 2002).

The ^{15}N NMR chemical shifts of N-7 in four QPA are summarized in Table 11. It is evident from the table that the substitution patterns on rings A and D have no effect on the shielding of the N-7 atom. However, the reduction of ring C (dihydroderivative) disturbs its aromatic nature and has a crucial effect on the isotropic shielding of the nitrogen nucleus. Moreover, nitrogen shielding is influenced by the electronic properties of the substituent at position C-8 (see dihydroberberines in Table 11).

5.4. X-ray diffraction analysis

Single-crystal X-ray diffraction is a precise method suitable for studying the bond lengths, bond and torsion angles, intermolecular interactions, and complete topology of crystal systems (Dostál et al., 2004; Man et al., 2001a,b; Marek et al., 2003a; Kariuki, 1995). Selected crystal data (bond lengths and valence angles) of the quaternary protoberberines and their 7,8-dihydroderivatives are summarized in Table 12. The table emphasizes the interatomic distances and bond angles that are somehow related to the iminium N7–C8 bond because this bond is the center of the principal reactivity of the QPA.

Table 9
^{1}H NMR chemical shifts (δ in ppm) of selected QPAs

Atom	Berberine	Palmatine	Jatrorrhizine	Pseudopalmatine	Caseadine	Columbamine	Thalifendine	Fissiaine	Dehydrocorydaline
H-1	7.79	7.75	7.33	7.45	–	7.52	7.76	7.29	7.56
H-4	7.09	7.15	6.50	6.72	–	6.80	7.07	–	7.06
H-5	3.22	3.25	3.07	3.15	3.18	3.30	3.19	3.23	3.20
H-6	4.95	4.95	4.79	5.00	4.74	4.97	4.89	4.88	4.94
H-8	9.91	9.95	9.50	10.34	9.16	9.45	9.69	9.63	9.82
H-9	–	–	–	7.67	–[b]	–	–	–	–
H-10	–	8.10	–	–	–	–	–	–	–
H-11	8.20	8.15	7.96	–	–	7.58	7.84	7.77	8.05
H-12	8.01	–	7.85	7.45	–[b]	8.11	7.84	7.90	8.17
H-13	8.96	9.15	8.44	8.40	9.08	8.45	8.83	8.77	–
2-OR	6.17	3.90	3.89	4.13	–[b]	–[a]	6.16	4.01	–[b]
3-OR		3.88	–[a]	4.09	–[b]	3.98		3.89	–[b]
9-R/10-R	4.10	4.15	4.06	4.06	–[b]	4.08	4.06	4.15	–[b]
10-R/11-R	4.07	4.00	4.16	3.98	–[b]	4.08	–[a]	–[a]	–[b]
13-OMe	–	–	–	–	–	–	–	–	2.50
Solvent	DMSO-d_6	DMSO-d_6	CD_3OD	$CDCl_3$	$CDCl_3$	CD_3OD	DMSO-d_6	CD_3OD	DMSO-d_6
References	Blaskó et al. (1988)	Wafo et al. (1999)	Kim et al. (2000)	Patra et al. (1987)	Suau et al. (1993)	Hsieh et al. (2004)	Chudík et al. (2006)	Chia et al. (1998)	Miyazawa et al. (1998)

[a] Not included in original report.

[b] NMR chemical shifts have not been assigned to the individual atoms: Caseadine 7.45, 7.30, 7.00, 4.14, 4.00; Dehydrocorydaline 4.12, 4.12, 4.07, 3.91.

Table 10
^{13}C NMR chemical shifts (δ in ppm) of QPAs

Atom	Berberine	Palmatine	Pseudopalmatine	Columbamine	Jatrorrhizine	Berberrubine	Thalifendine	Dehydrocorydaline
C-1	105.43	109.70	105.1	114.9	110.9	105.1	105.18	111.6
C-2	147.63	150.80	152.6	143.7	149.4	[b]	147.53	146.5
C-3	149.76	153.07	149.2	150.4	146.4	[b]	149.40	150.1
C-4	108.43	112.00	111.6	109.4	116.3	108.6	108.30	112.4
C-4a	130.64	129.80	129.2	133.6	130.2	[b]	130.17	133.3
C-5	26.35	27.80	27.2	26.3	29.1	27.5	26.33	26.0
C-6	55.19	56.50	57.1	55.6	57.6	56.3	54.93	55.6
C-8	145.42	146.20	144.1	144.8	145.2	144.4	143.55	145.2
C-8a	121.37	124.30	123.2	117.4	119.2	[b]	122.09	119.1
C-9	143.61	145.6	109.3	148.0	151.5	[b]	141.11	150.8
C-10	150.36	151.5	139.5	149.9	145.1	[b]	150.88	143.8
C-11	126.67	128.00	153.7	126.5	122.9	[b]	123.34	127.0
C-12	123.53	–[a]	106.4	123.3	125.7	[b]	123.42	123.4
C-12a	132.94	135.4	123.1	128.2	128.2	[b]	131.58	128.5
C-13	120.18	121.1	118.3	119.8	119.3	[b]	120.17	119.5
C-13a	137.41	139.9	138.0	138.3	134.9	137.6	136.01	137.9
C-13b	120.42	120.4	119.3	121.4	120.9	[b]	120.62	121.4
C2-R	102.07	57.3	[c]	–	58.3	102.4	101.89	56.0
C3-R		56.8	[c]	56.9	–			57.2
C9-R/C10-R	61.95	62.4	[c]	61.8	63.0	–	60.96	61.9
C10-R/C11-R	57.06	57.4	[c]	56.3	57.0	57.1	–	56.0
C13-OMe	–	–	–	–	–	–	–	39.8
Solvent	DMSO-d_6	DMSO-d_6	$CDCl_3$	$CDCl_3$ + DMSO-d_6	$CDCl_3$ + DMSO-d_6	$CDCl_3$	DMSO-d_6	DMSO-d_6
References	Blaskó et al. (1988)	Wafo et al. (1999)	Suau et al. (1991)	Rasoanaivo et al. (1991)	Rasoanaivo et al. (1991)	Suau et al. (1991)	Chudík et al. (2006)	Miyazawa et al. (1998)

[a] Not included in original report.

[b] NMR chemical shifts have not been assigned to the individual atoms: 143.2, 145.4, 149.0, 151.2 (C-2, C-3, C-9, C-l0), 119.1, 120.1, 125.0 (C-11, C-12, C-13), 117.1, 129.6, 132.7 (C-4a, C-8a, C-12a, C-13b).

[c] NMR chemical shifts have not been assigned to the individual atoms: 56.9, 56.8, 56.3, 55.8.

Table 11
^{15}N NMR chemical shifts (δ in ppm)[a] of QPAs in DMSO-d_6 (D) and 8-substituted-7,8-dihydroberberines in CD_2Cl_2 (C) at 303 K (Marek et al., 2003b, 1999, 2002)

Alkaloid/derivative	Chemical shift
Berberine	194.8 (D)
Coptisine	194.9 (D)
Palmatine	194.5 (D)
Jatrorrhizine	194.4 (D)
8-Hydroxy-7,8-dihydroberberine	88.5 (C)
8-Methoxy-7,8-dihydroberberine	83.6 (C)
8-Trichloromethyl-7,8-dihydroberberine	64.9 (C)

[a] Referenced relative to liquid ammonia (Marek and Lyčka, 2002).

The interatomic distance N7–C8 in quaternary protoberberines lies in the range 132–134 pm. This bond is somewhat longer in the 8-oxoderivatives (139 pm) and reaches 144–145 pm in the dihydroderivatives (products of nucleophilic addition to the QPA skeleton; see Chapter 6). Similarly, C8–C8a gets longer in the order QPA (138–140 pm), 8-oxoderivatives (147 pm), dihydroderivatives (149–151 pm). In the same order of derivatives, the bond angle N7–C8–C8a is reduced (120–122°, 115–117°, 109–110°).

Quaternary protoberberine cations are relatively planar species. Their planarity is disturbed only in the partially saturated ring B, which adopts a twisted half-chair conformation with the atoms C5 and C6, deviating from the plane of rings A, C and D. In 8-substituted-7,8-dihydroderivatives (adducts) the ring C also becomes partially saturated, forming a shallow half-chair with the C-8 atom (in sp^3 hybridization) deviating. The group attached at C-8 is usually in the pseudoaxial position. The methoxy group at C-10 is positioned in-plane while the neighboring 9-OMe is almost perpendicular to the plane of the D-ring. This is a common feature of the tertiary 8-adducts, 8-oxoderivatives, and quaternary protoberberine salts. The interplanar angles between the aromatic rings of some QPA and derivatives are given in Table 13.

The crystal structures of the compounds studied provided a number of interesting findings on non-covalent intermolecular interactions. In QPA salts, cations are packed in columns with the spaces between occupied by anions (Kariuki, 1995). Berberine formate–succinic acid displayed non-conventional C–H···O hydrogen bonds (Marek et al., 2003a). Four molecules of 8-hydroxydihydroberberine were linked by four hydrogen bonds to make an eight-membered heterocycle with alternating H and O atoms (Dostál et al., 2004). Also in 8-aminodihydroberberine, four molecules were associated with six H-bonds (Man et al., 2001a).

6. Properties and reactivity

Quaternary protoberberine alkaloids are brightly colored solids. Phenolic QPA typically change the color upon changes in the pH of their solution (Shamma, 1972). For example, the alkaloid jatrorrhizine is yellow in a neutral solution, but turns to deep red in an alkaline environment. Columbamine, which is also yellow in neutral solution, becomes only tan in hydroxide solution. The bright colors of QPA can be employed advantageously in monitoring the progress of a column-chromatography separation. Quaternary protoberberines without a phenolic group change their bright colors from the typical yellow to very slightly colored or even colorless substances.

As mentioned previously, quaternary protoberberine alkaloids are derivatives of 5,6-dihydrodibenzo[*a*,*g*]quinolizinium. These iminium cations are characterized by the sensitivity of the polar $C{=}N^+$ bond to a nucleophilic attack followed by the formation of adducts with substituents at position C-8 (Marek et al., 2003b). Strong base is capable of converting the quaternary alkaloid to the corresponding base (for p*K* values of QPA, see Table 14). In contrast to the majority of classical alkaloids, however, the bases possess a non-classical structure with an OH group covalently bonded to carbon C-8 (Scheme 6).

Berberine chloride was converted to its base using a 10–20% solution of NaOH in water. When the product was extracted into dichloromethane, 8-hydroxy-7,8-dihydroberberine, formed by the addition of OH^- to the C-8 of the iminium moiety, was identified using NMR as a major component of the solution. In addition, the bimolecular aminoacetals (both diastereomers), formed by the condensation reaction between two molecules of the base, were detected as minor forms in the organic solvent (Scheme 7) (Dostál et al., 2004). However, these basic forms are very unstable compounds and traces of acid immediately convert them back to the salts.

6.1. Nucleophilic addition

6.1.1. O-Nucleophiles

The nucleophilic addition of OR^- to the berberine skeleton is a simple analogy to the formation of a berberine base in alkaline solution. The 8-methoxy and 8-ethoxy-7,8-dihydroderivatives prepared by the reaction of QPA with NaOR (Scheme 8) are unstable compounds, easily converted back to the quaternary forms in an acidic environment. From time to time, 7,8-dihydroprotoberberines have been reported as genuine natural products (Wafo et al., 1999; Li et al., 2001). However, due to the acidic nature of the plant tissues, 8-hydroxy-, 8-methoxy- and 8-ethoxy-7,8-dihydroprotoberberines must be classified as isolation artifacts. They are formed during the isolation or separation procedures in alkaline media in the presence of common solvents (methanol, ethanol) (Marek et al., 2003b).

6.1.2. N-Nucleophiles and S-nucleophiles

In 2001, Man and co-workers (Man et al., 2001) tried to prepare 8-substituted-7,8-dihydroberberines by reacting the quaternary berberine with azide and thiocyanate.

Table 12
Bond lengths (Å) and bond angles (°) of selected QPAs and 7,8-dihydroprotoberberine derivatives

Alkaloid	R-factor	N7-C6	N7-C8	N7-C13a	C8-C8a	C8-R	C6-N7-C8	N7-C8-C8a	C8-N7-C13a	CDC code	Refs.
Berberine chloride tetrahydrate	7.01	1.481	1.337	1.378	1.403	–	117.67	121.72	121.67	YUJHAM	1
Berberine chloride ethanol solvate hemihydrate	5.62	1.491	1.316	1.394	1.405	–	118.09	122.08	122.61	YUJHIU	1
Berberine bromide dihydrate	4.71	1.486	1.320	1.394	1.396	–	118.57	121.09	123.18	YUJHOA	1
Berberine iodide	6.66	1.501	1.316	1.381	1.393	–	117.58	121.81	123.61	YUJHUG	1
Berberine hydrogen sulfate	6.10	1.499	1.324	1.398	1.393	–	118.17	122.00	122.40	CISREB	2
bis(Berberine) sulfate heptahydrate[a]	7.64	1.472	1.341	1.373	1.397	–	119.14	123.22	122.01	YUJJAO	1
Berberine azide acetonitrile solvate	6.29	1.492	1.334	1.390	1.398	–	117.50	121.87	122.29	UCACUW	3
Berberine thiocyanate	8.29	1.484	1.332	1.389	1.401	–	118.59	121.42	122.48	UCADAD	3
Berberine formate succinic acid	3.40	1.499	1.338	1.399	1.404	–	118.18	121.47	122.34	OLOFUQ	4
Palmatine iodide	4.20	1.483	1.340	1.402	1.381	–	117.93	122.00	121.81	QEMYUC	5
Palmatine chloride phenol solvate monohydrate	5.50	1.500	1.340	1.385	1.399	–	117.35	120.93	122.38	FINPUN	5
Jatrorrhizine chloride dihydrate	5.80	1.499	1.337	1.389	1.407	–	117.63	121.32	122.39	SOHNIM	5
Jatrorrhizine chloride dihydrate	5.70	1.495	1.323	1.390	1.398	–	118.84	122.29	122.42	SOHNIM01	6
Dehydrocorydaline hydrochloride hydrate	5.30	1.476	1.324	1.404	1.401	–	117.29	121.36	122.90	CEXNUO	5
8-Hydroxy-7,8-dihydroberberine	8.91	1.463	1.444	1.400	1.494	1.440	119.46	109.95	120.55	AMIZIF	7
8-Methoxy-7,8-dihydroberberine	5.19	1.465	1.437	1.410	1.504	1.460	116.41	110.63	119.47	BAPVAQ	8
8-Amino-7,8-dihydroberberine	8.75	1.464	1.473	1.415	1.512	1.454	113.34	109.87	117.40	/	9
8-Amino-7,8-dihydrocoptisine	5.72	1.453	1.468	1.394	1.502	1.465	115.37	108.86	118.98	/	9
8-Cyanodihydroberberine	4.36	1.462	1.451	1.416	1.515	1.508	114.13	110.65	115.94	UCADEH	3
8-(Trichloromethyl)-7,8-dihydroberberine	6.60	1.467	1.441	1.388	1.511	1.589	119.41	109.97	120.60	RENMUS	10
8-(Trichloromethyl)-7,8-dihydroberberine	3.17	1.463	1.443	1.398	1.515	1.584	119.33	110.07	120.22	RENMUS01	8
8-(Trichloromethyl)-7,8-dihydropalmatine	3.53	1.464	1.450	1.400	1.509	1.577	118.70	112.31	119.85	BAPVEU	8
8-Oxoberberine	3.37	1.475	1.388	1.395	1.474	1.230	115.93	116.68	123.99	AMIZUR	7
8-Oxocoptisine	3.31	1.476	1.388	1.404	1.470	1.235	117.37	115.41	124.34	AMIZOL	7
12,13-Dinitro-oxyberberine[a]	4.00	1.473	1.392	1.393	1.473	1.221	115.62	116.49	123.27	SIKGIC	11

1. Kariuki (1995), 2. Abadi et al. (1984), 3. Man et al. (2001b), 4. Marek et al. (2003a), 5. CCDC (1999) 6. Ghosh et al. (1993), 7. Dostál et al. (2004), 8. Marek et al. (2003b), 9. Man et al. (2001a), 10. Khamidov et al. (1996), 11. Cushman et al. (1990).

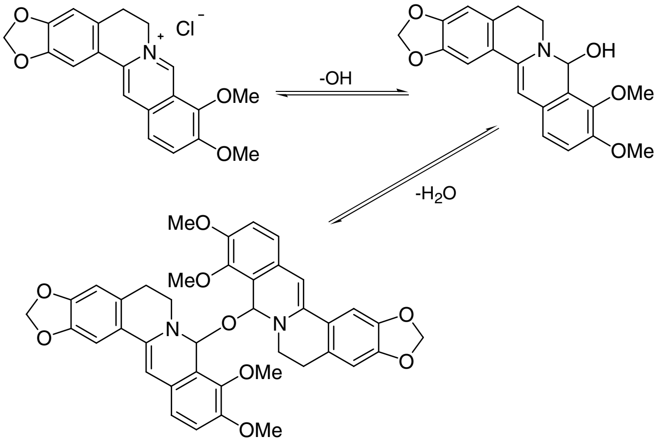
[a] Average values for two symmetry independent molecules.

Table 13
Interplanar angles (°) in selected compounds derived from berberine

Compound	A/C	A/D	C/D	Refs.
Berberine formate	13.36	13.22	1.69	Marek et al. (2003a)
Berberine azide	11.02	10.10	0.93	Man et al. (2001b)
Berberine thiocyanate	11.02	10.10	0.93	Man et al. (2001b)
8-Hydroxydihydroberberine	21.50	31.47	13.06	Dostál et al. (2004)
8-Aminodihydroberberine	23.03	25.58	8.76	Man et al. (2001a)
8-Cyanodihydroberberine	29.45	37.69	14.09	Man et al. (2001b)
8-Trichloromethyldihydroberberine	39.58	45.26	14.64	Marek et al. (2003b)

Table 14
pK values of QPAs (Šimánek et al., 1976) obtained for methanolic solutions

Alkaloid	pK
Dihydrodibenzo[*a*,*g*]quinolizinium	14.3
Palmatine	15.7
Berberine	15.4
Coptisine	13.8
Corysamine	15.2
13-Methylberberine	16.5
13-Methoxyberberine	16.4



Scheme 7.

Under the experimental conditions described, however, the reaction did not afford the expected products. Instead, berberine azide and berberine thiocyanate were isolated from the reaction mixture. In contrast, 8-aminoderivatives were formed during the reaction of a QPA and a primary amine in ethanol or methanol (Naruto et al., 1976). The reaction of berberine and coptisine with liquid ammonia produced 8-aminoderivatives (Man et al., 2001). Further, the preparation of adducts with an exocyclic C8=NR double bond (Bhakuni and Jain, 1986) and the preparation of 7,8-dihydroberberines with amine (–NH–R), hydrazine (–NH–NRR′) or hydroxylamine (–NH–OR) groups attached to the C-8 has been published (Möhrle and Biegholdt, 1982).

To the best of our knowledge, the preparations or structural characterizations of any covalent adduct with an S-nucleophile has not yet been reported.

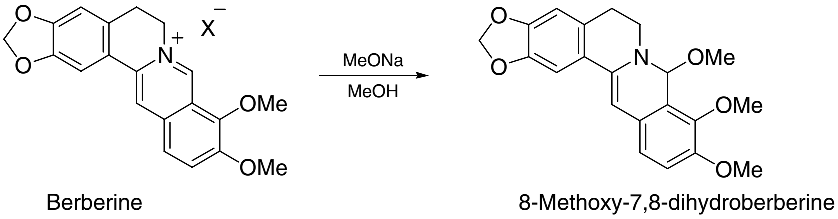
6.1.3. *C-Nucleophiles*

The sensitivity of atom C-8 in the polarized N7=C8 iminium bond to nucleophilic attack has allowed the condensation of berberine with phenyl- or benzylmagnesium bromide (Scheme 9).

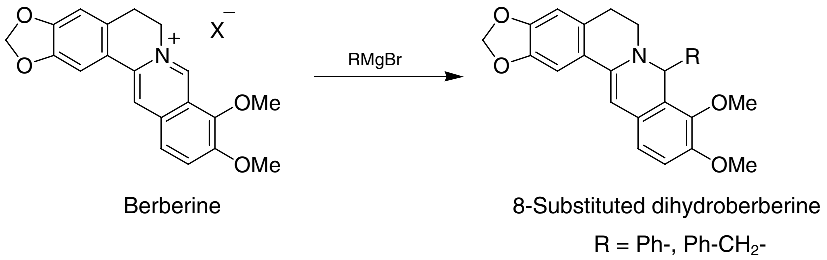
Reacting berberine with benzylmagnesium bromide produced 8-benzyl-7,8-dihydroberberine (Shamma, 1972). Reduction of the product with sodium borohydride yields 8-benzylcanadine, which can undergo cleavage of ring C through Hofmann degradation (Scheme 10).

Another possibility for incorporating a carbon atom at position C-8 of a QPA is reaction with chloroform (Scheme 11). This reaction produces 8-trichlormethyl-7,8-dihydroberberine (Marek et al., 2003b; Shamma and Rahimizadech, 1986; Miana, 1973; Slavík and Slavíková, 1989).

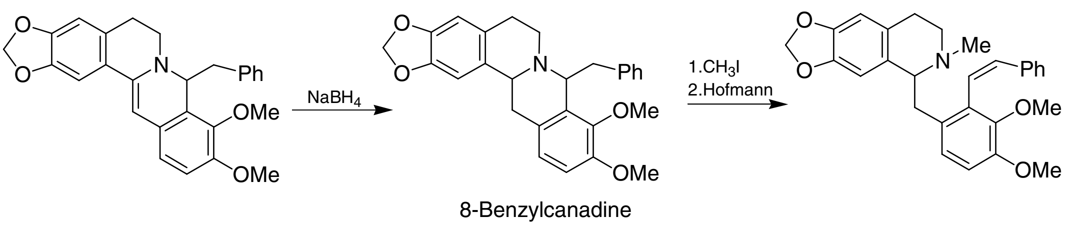
Generally, when chloroform is used for separating the QPA from plant material under alkaline conditions, 8-trichloromethyl derivates are easily formed. In the past, these 8-trichloromethyl products have been incorrectly characterized as protoberberine bases (8-hydroxy derivatives) (Wafo et al., 1999; Li et al., 2001). Obviously, these compounds are isolation artifacts (Marek et al., 2003b).



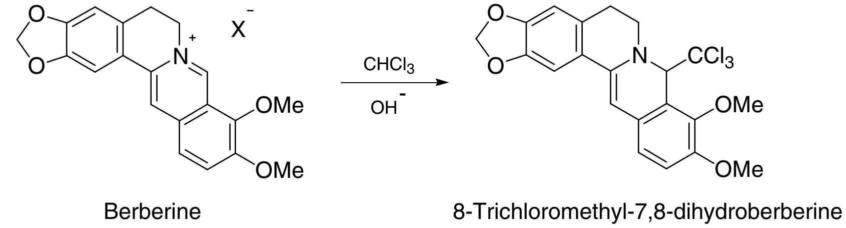
Scheme 8.



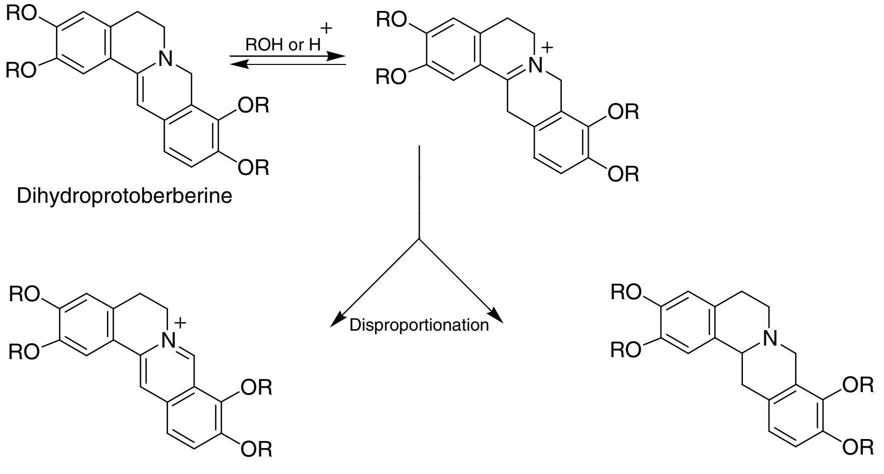
Scheme 9.



Scheme 10.



Scheme 11.

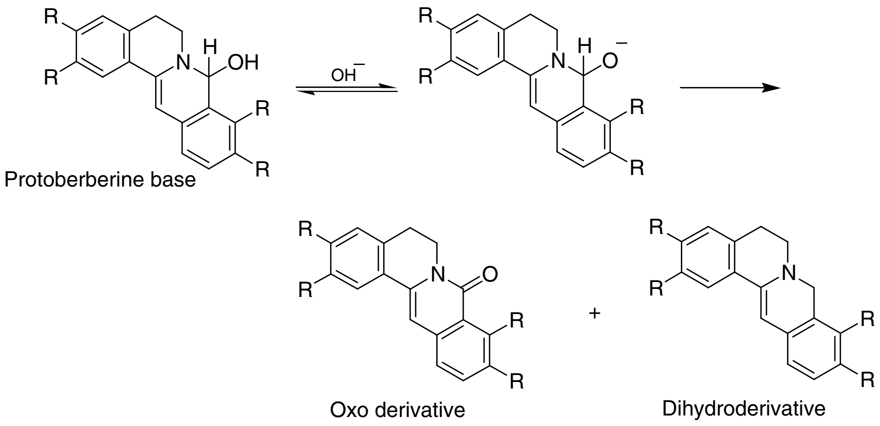


Scheme 12.

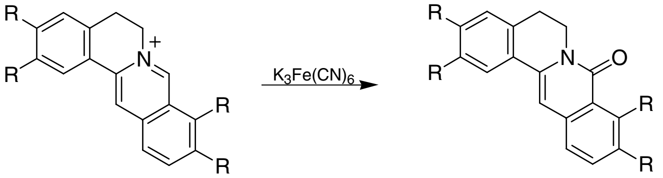
However, compounds of this type must be characterized carefully because their identification is not always straightforward due of the lack of protons in the –CCl_3 substituent (^{1}H NMR), the easy splitting of the C8–CCl_3 bond (mass spectrometry), and the greatly reduced intensity of the –CCl_3 signal in ^{13}C NMR spectra (long relaxation time or inefficient NOE).

6.2. Disproportionation

In a protic medium, a dihydroprotoberberine derivative is in equilibrium with its iminium form (Scheme 12). The latter species is unstable and undergoes rapid disproportionation to form a mixture of the quaternary protoberberine salt and the tetrahydroprotoberberine.



Scheme 13.



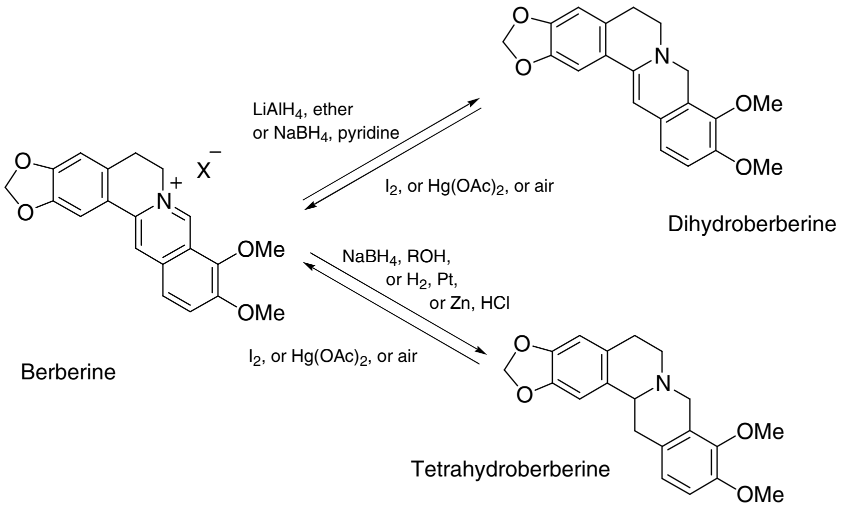
Scheme 14.

Quaternary protoberberine salts are unstable in the presence of concentrated alkali. The alkaloid bases formed under basic conditions are subsequently transformed into the 8-oxo-7,8-dihydro- and 7,8-dihydroderivatives (Scheme 13) (Shamma, 1972).

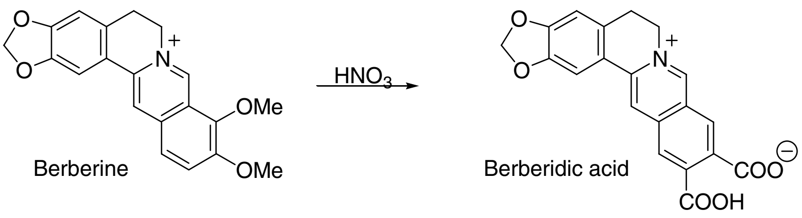
6.3. Oxidation and reduction

The 8-oxoderivative can be obtained quantitatively from the QPA by oxidation with potassium ferricyanide (Scheme 14).

Quaternary protoberberine salts can be reduced (Shamma, 1972) to the corresponding tetrahydroprotoberberines with a variety of reducing agents (Scheme 15) (Bhakuni and Jain, 1986). When mixed metal hydride in



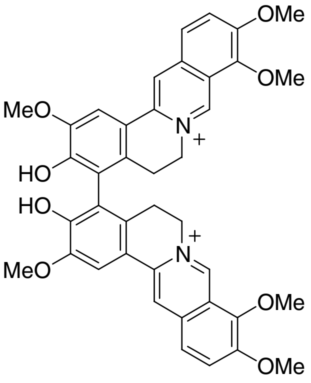
Scheme 15.



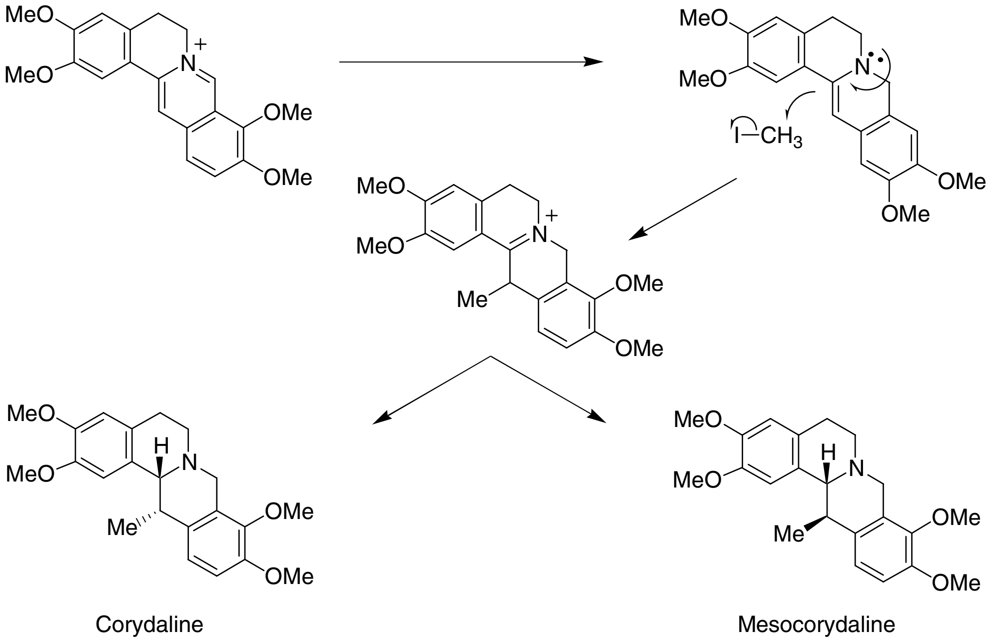
Scheme 16.

a dry aprotic solvent is used for the reduction, the reaction is stopped at the dihydroprotoberberine stage. Re-oxidation of the dihydro- or tetrahydroprotoberberine to form the quaternary salt can be accomplished with iodine (Cava et al., 1968), mercuric acetate, or simply by standing in air (Meise, 1971). The redox reaction of berberine on a mercury electrode (Komorsky-Lovrić, 2000) is totally irreversible and occurs between −0.95 and −1.35 V, depending on the berberine concentration and the pH of the solution.

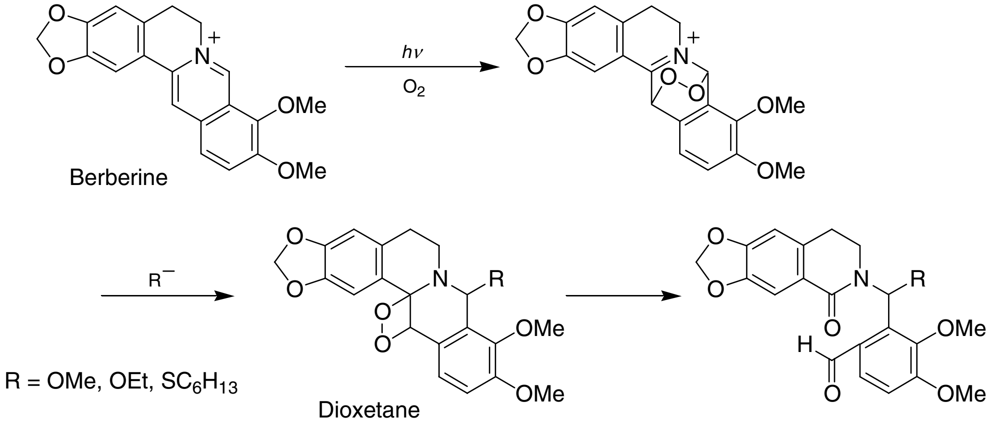
Oxidation of a quaternary protoberberine salt with hot dilute nitric acid yields a golden betaine. In the case of berberine, the product is presumably the betaine berberidic acid, which has been formulated as indicated below (Scheme 16) (Shamma, 1972; Bhakuni and Jain, 1986).



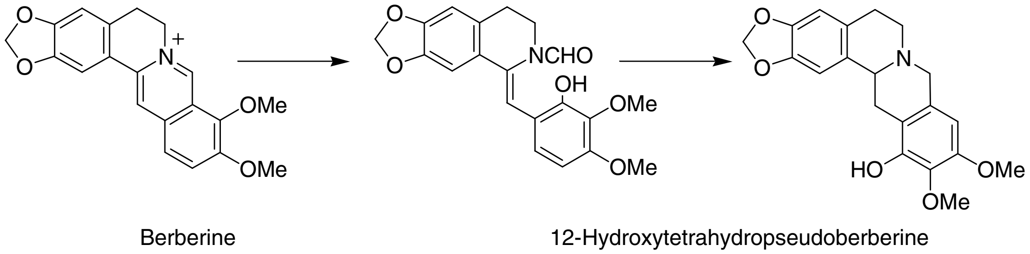
Scheme 17.



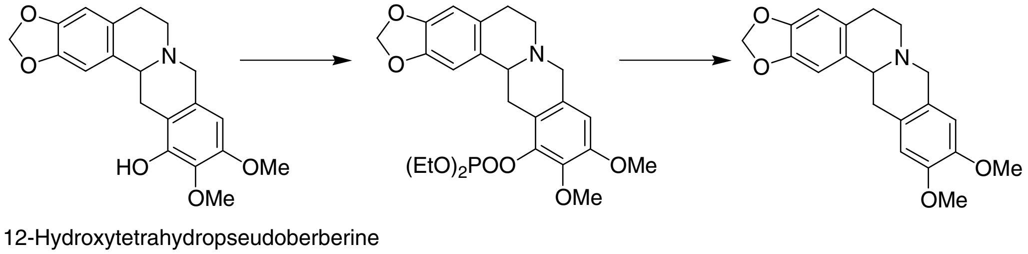
Scheme 18.



Scheme 19.



Scheme 20.



Scheme 21.

Bisjatrorrhizine (Scheme 17), obtained from *Jatrorrhiza palmata*, is formally produced by the ortho oxidative coupling of the phenolic group of jatrorrhizine (Bhakuni and Jain, 1986; Fajardo et al., 1996).

6.4. Alkylation at position C-13

The conversion of palmatine to corydaline and mesocorydaline (Scheme 18) via an intermediate is an early example of the currently well-known *C*-alkylation of an enamine (Jeffs, 1967). The relative proportion of corydaline to mesocorydaline obtained from the intermediate salt apparently varies with the nature of the reducing agent; zinc–acid systems afford predominantly corydaline while borohydride reduction is claimed to give corydaline exclusively.

6.5. Photochemical reactions

Photochemical reaction (Morrison, 1990) has been proved to be an important pathway for the transformation of myriad molecules in biological systems. The photochemistry of protoberberines (Contreras et al., 1984) is currently of great interest to molecular biologists and biochemists investigating biologically relevant samples containing protoberberines (see Chapter 7). Very recently, berberine has been indicated as a photosensitizer in the oxidation of guanine to the 8-oxoguanine (Hirakawa et al., 2005).

The Rose-Bengal sensitized photooxygenation of berberine and also dihydroberberine gives the corresponding berberinephenolbetaine in good yields. In the absence of any sensitizer the photochemical oxidation of dihydroberberine produces mainly berberine. The reaction of berberine does not proceed under similarly unsensitized conditions unless sodium methoxide or an other nucleophile is present in the reaction mixture. Under those conditions the lactamic aldehyde is formed (Scheme 19).

8-Hydroxymethylberberines can be prepared from the corresponding quaternary protoberberines by a photochemical route. The reaction mechanism includes two subsequent steps – chemical sensitization (acetone, benzophenone) and single electron transfer (Suau et al., 1999a,b). For other photochemical reactions, see the review by Singh et al. (1980).

6.6. Transformation of 2,3,9,10-substituted QPA to 2,3,10,11-substituted QPA

The 2,3,10,11-tetrasubstituted QPA are sometimes called pseudoberberines (see Chapter 1.2). Their formation from the 2,3,9,10-substituted analogs was published in 1985 (Hanaoka et al., 1985). Naturally occurring 2,3,10,11-substituted QPA were synthesized from the corresponding 2,3,9,10-substituted QPA through oxidative C8–C8a bond cleavage, photocyclization, and subsequent deoxygenation.

Berberine was oxidized with *m*-chloroperbenzoic acid in dry tetrahydrofuran in the presence of sodium hydride to afford an open intermediate in 76% yield (Scheme 20). This intermediate had previously been synthesized from berberine by a similar oxidation using sodium bicarbonate instead of sodium hydride, however, in only 20% yield. The photocyclization reaction of the enamide intermediate in ethanol was done under a stream of nitrogen using a high-pressure mercury lamp. Subsequent reduction using sodium borohydride produced 12-hydroxytetrahydropseudoberberine (yield 79%).

Treatment of 12-hydroxytetrahydropseudoberberine with diethyl chlorophosphate in the presence of sodium hydride afforded the phosphate, hydrogenolysis of which using sodium in liquid ammonia (at −70 °C) produced tetrahydropseudoberberine (53%) (Scheme 21).

Beside the transformation of 2,3,9,10-substituted QPA to 2,3,10,11-tetrasubstituted QPA, transformations of QPA can produce structurally related benzo[*c*]phenanthridines (Iwasa et al., 1989; Iwasa and Kamigauchi, 1996; Jeffs and Scharver, 1976; Iwasa and Kim, 1997; Hanaoka et al., 1986). Conversions of berberine, (Moniot and Shamma, 1979; Elango and Shamma, 1983), karachine (Blaskó et al., 1982), and berberrubine (Iwasa and Kamigauchi, 1996; Das and Srinivas, 2002) have been published.

7. Biological activities

Phytomedicine (Tsai and Tsai, 2004), including natural products from traditional herbal medicines used for medical and health-fortifying purposes, is gaining international popularity. Extracts of *Berberis aristata* and *Coptis chinensis* have been used in traditional Oriental medicine for the treatment of gastroenteritis and secretory diarrhea. It is reasonable to presume that the quaternary protoberberines belong to the biologically active constituents of these plants.

Protoberberine alkaloids display a great variety of biological and pharmacological activities. These activities include the inhibition of DNA synthesis (Schmeller et al., 1997), protein biosynthesis, the inhibition of membrane permeability, and the uncoupling of oxidative phosphorylation. These processes likely contribute to the allelochemical and toxic effects observed against bacteria, fungi, other plants, insects, and vertebrates. Interaction with DNA and inhibition of reverse transcription could be responsible for the inhibition of phages and other viruses. The interactions with neuroreceptors, the inhibition of ATPase, and the binding to microtubules should affect mainly insects and vertebrates. However, only some representative examples of the biological effects of QPA are mentioned briefly in the following paragraphs. The number of biological studies amounts to hundreds of papers published annually.

7.1. Interactions with biomacromolecules – structural studies

The investigation of the interactions of QPA with biomacromolecules at the atomic level and the structural description of their complexes represent key steps in the real understanding of the biological function of the QPA. This research field has been developing dynamically during the past few years as documented by the number of papers published annually. The development is to a large extent driven by the increasing number of sophisticated instrumental techniques, including nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS), capillary zone electrophoresis, and many others.

The ability of the protoberberines to act as a poison against Topoisomerase-I (Topo-I) and Topoisomerase-II (Topo-II), has been related to their antitumor activity (Kim et al., 1998; Mazzini et al., 2003). The inhibitors of the enzyme Topo-II have been studied more frequently than those of Topo-I and the principal mechanisms of inhibition of Topo-II have been discovered. However, the role of the drug–DNA interactions in Topo-I inhibition is still unclear, although the binding of the protoberberine to DNA has been considered to be responsible for this activity.

Three principal noncovalent modes of binding small ligands to oligonucleotides can be distinguished: (a) external binders, often polyamines that make nonspecific electrostatic contacts with the the DNA backbone; (b) minor groove binders, the most sequence-selective class, thanks to a network of specific H-bonds to base-pairs and backbone functions; and (c) base-pair intercalators which establish extended and partly specific, van de Waals contacts with the floors of aromatic pairs (Micco et al., 2006). The interaction of berberine with several oligonucleotides, studied by NMR spectroscopy, showed that berberine binds preferentially to AT-rich sequences (Mazzini et al., 2003). 2D NOE experiments enabled the detection of several contacts between protons of berberine and protons of the self-complementary oligomer $d(AAGAATTCTT)_2$. The berberine molecule is located in the minor groove of the double helix of the nucleotide at the level of the A_4–T_7 and A_5–T_6 base pairs. It lies with the convex side on the helix groove, thus presenting the positive nitrogen atom of the alkaloid close to the negative ionic surface of oligonucleotide. Ring A and the methylendioxy group are external to the helix, while the aromatic protons H-11 and H-12 are close to the ribose of cytidine C_8.

The noncovalent complexes of several protoberberine alkaloids (berberine, palmatine, jatrorrhizine, and coptisine) have been investigated by using electrospray ionization mass spectrometry (ESI-MS) (Chen et al., 2004). The results indicated that palmatine exhibited the greatest binding affinity with the double-stranded DNA, while berberine had the lowest affinity. The preliminary results indicated that the berberine showed some sequence selectivities. Other ESI-MS studies of the noncovalent complexes have shown several new results (Chen et al., 2005b). Five cytotoxic protoberberine alkaloids, berberine, palmatine, jatrorrhizine, coptisine, and berberrubine were tested with a few double stranded oligonucleotides. The different oligonucleotides gave the different orders of the relative binding affinity. The results from ESI-MS and fluorescence titration experiments indicated that sequence selectivity of these five alkaloids was not significant and no remarkable AT- or GC-rich DNA binding preference was obtained. This is in clear contrast to the above-mentioned reports (Chen et al., 2004; Mazzini et al., 2003) describing the preferential binding of berberine to AT-rich DNA.

Characterization of the quaternary berberines modified at the position C-9 showed that the berberine derivatives, especially those with a primary amino group, strongly bind with calf-thymus DNA, presumably via an intercalation mechanism (Pang et al., 2005). Structure–activity relationships of the protoberberine analogs demonstrate that substitution at the C-9 position is an important determinant of the biological activity (Park et al., 2004).

Studies of bridged berberine derivatives (two berberine units bridged at the position C9 with different linker lengths) showed that compounds with a propyl chain exhibit the highest binding affinity to DNA (Chen et al., 2005a; Qin et al., 2006).

The results of binding sanguinarine and berberine to triple and double helical DNA and RNA structures show that both alkaloids can bind and stabilize the DNA and RNA triplexes more strongly than their respective parent duplexes (Das et al., 2003).

The interaction of QPA with DNA was investigated using capillary zone electrophoresis (Vlčková et al., 2005). In this experiment two QPA were studied. Ethidinium bromide, a classic DNA intercalator, was used as a reference. The results indicated that these alkaloids do not attach covalently to DNA constituents.

7.2. Cytotoxic activity and apoptosis

Probably the most important and most frequently tested property of the QPA is cytotoxicity (Sanders et al., 1998; Orfila et al., 2000; Colombo et al., 2001; Chen et al., 2005). The cytotoxic activity of the QPA seems to be mainly due to apoptosis and inhibition of telomerase.

Berberine was tested for its potential inhibitory effect against telomerase activity (Meyerson, 2000) on a human leukemia cell line (Naasani et al., 1999). It was identified as a moderate inhibitor with 50% inhibition at 35 μM concentration. Results obtained on telomerase of *Plasmodium falciparum* (Sriwilaijareon et al., 2002) showed, that the telomerase is sensitive to inhibition by the berberine in a range of 30–300 μM.

The high concentration of berberine (75 μg/ml) induced an acute cytotoxic activity (Letašiová et al., 2005). Berberine derivatives were identified as potential inhibitors of caspase 3, a major apoptosis effector. The inhibitory effect was determined at 20 μM concentration of ligand (Letašiová et al., 2005; Kim et al., 2002).

A comparative study of the inhibitory and anti-leukemic activities of some protoberberine and benzo[*c*]phenanthridine alkaloids showed that the activities of the QPA are lower than those of the benzophenanthridines (Sethi, 1985).

The cytotoxic activity of protoberberine was tested predominantly in vitro (Iwasa et al., 2001). Extensive experiments on twenty four quaternary protoberberine alkaloids were evaluated on thirty eight human cancer cell lines. Six compounds were cytotoxic and some others exhibited lower levels of cytotoxicity. From a structure–activity point of view, some trends were observed. For berberine and palmatine derivatives bearing a 13-alkyl side chain, the cytotoxicity increased parallel to the number of CH_2 units in the side chain. 12-Bromo-8-hexylberberine was more cytotoxic than the corresponding 8-phenyl and 8-butylderivatives, suggesting that the length of the carbon unit at C-8 also influences the cytotoxicity. Bromination at C-12 increased the cytotoxicity (Iwasa et al., 2001).

The 8-oxoderivatives of the protoberberine alkaloids were tested for cytostatic activity in vitro using MDA-MB-231 mammary tumor cells (Weimar et al., 1991). Tetramethoxy-8-oxoberberine inhibited cell proliferation at a concentration of 10^{-5} M, its cytostatic effect did not depend on intercalation into DNA.

The antimutagenic potency of berberine and jatrorrhizine was evaluated against acridine orange (AO) by using *Euglena gracilis* as a eucaryotic test model (Černáková et al., 2002). Both alkaloids showed significant concentration-dependent inhibitory effect against the AO-induced chloroplast mutagenesis of *E. gracilis*. The quaternary benzo[*c*]phenanthridine alkaloids tested did not exhibit the same effect.

Slaninová et al. investigated the effects of berberine, coptisine, and some benzo[*c*]phenanthridine alkaloids on the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces japonicus*. The protoberberine alkaloids exhibited some effects; however, these effects were significantly smaller than those of the benzophenanthridines (Slaninová et al., 2001).

7.3. Antimicrobial activity

Berberine, jatrorrhizine, and the crude extract of *Mahonia aquifolium* have shown strong activity against twenty clinical isolates of *Propionibacterium* acnes with minimal inhibitory concentration values (Slobodníková et al., 2004). Berberine seems to be more active than jatrorrhizine against coagulase-negative staphylococci. The antifungal activity tested against *Candida* showed that only *C. tropicalis* (resistant to nyastin, miconazole, and econazole) was strongly inhibited by all of the agents tested.

Experiments with the 8-alkyl and 8-phenyl-substituted berberines and their bromo derivatives showed that the introduction of hydrocarbon groups at position C-8 increased the antimicrobial activity (Iwasa et al., 1998). The 12-bromo derivatives of the 8-alkyl- and 8-phenyl-protoberberines showed higher activity against the microorganisms tested than did their non–brominated analogs.

Jatrorrhizine may serve as a leading compound for further studies to develop new antifungal agents with highly potent antifungal activity and low host toxicity (Volleková et al., 2003). The side carbon chain at position C-13 of quaternary protoberberines was investigated for its potential to increase the fungicidal and herbicidal activity (Iwasa et al., 2000). Parallel to the QPA, tetrahydroforms with methyl group at position C-13 were tested for in vitro and in vivo activities. Whereas the tertiary forms of alkaloids did not exhibit significant activity, a few of the quaternary forms showed some activity against *Leptosphaeria nodrum* and *Puccinia recongita*.

13-Hexylberberine and 13-hexylpalmatine showed the strong activity against *Staphylococcus aureus*, being more active than berberine and kanamycin sulfate. Both hexylderivatives possessed antifungal activity (Iwasa et al., 1997). Berberrubine, the isolation artifact, was found to be active against *Mycobacterium smegmatis* (Gharbo et al., 1973).

7.4. Anti-inflammatory activity

Extracts of the roots of berberine–containing Berberidaceae were tested in acute cases of inflammation (carrageenan- and zymosan-induced paw oedema in mice). The total ethanol extract showed a higher reducing effect as compared to the three alkaloid fractions and the major constituents berberine and oxyacanthine (Ivanovska and Philipov, 1996).

7.5. Antimalarial activity

It has been reported that berberine is a potent in vitro inhibitor of both nucleic acid and protein synthesis in human malaria *Plasmodium falciparum* FCR-3 (Elford, 1986; Vennerstrom and Klayman, 1988). In addition, the inhibitor activity against telomerase of human malaria *P. falciparum* has been presented (Sriwilaijareon et al., 2002).

In vitro structure–activity relationship studies for antimalarial activity against *P. falciparum* have been published (Iwasa et al., 1998, 1999). The type of the oxygen substituent on rings A, C, and D and the position of the oxygen functions on ring D influence the activity of the protoberberine alkaloids. Shifting the oxygen function from C-9 and C-10 to C-10 and C-11 results in a significant increase in activity.

7.6. Other effects

Berberine possesses an anti-diabetic effect (Leng et al., 2004), which is related to the property of stimulating insulin secretion and modulating lipids. The anti-arrhythmic activity (Yang and Wang, 2003) of a new derivate of berberine CPU-86017 (7-(4-chlorbezyl)-7,8,13,13a-tetrahydroberberine chloride) has been tested. Inhibitory effects of berberine on potassium and calcium currents in isolated rat hepatocytes may be involved in hepatoprotection (Tsai and Tsai, 2004; Wang et al., 2004a,b). Kinetics of alkaloid uptake to vacuoles has been described (Sato et al., 1993).

8. Conclusions and perspectives

In conclusion, quaternary protoberberine alkaloids represent a very interesting and significant group of natural products with a broad range of biological activities. Owing to the presence of the polarized iminium bond, positive charge, and relatively planar skeleton, QPA interact with a range of molecular and biological targets, including nucleic acids and proteins.

The isolation and purification procedures of QPA represent a relatively well established field. The classical isolation sequences are frequently being replaced by modern chromatographic techniques at micro-preparative and preparative scales. Parallel to the isolation of new structures from natural sources, modified berberine skeletons are constructed using modern synthetic methods in order to find highly biologically active substances.

Structural studies of complexes between protoberberine alkaloids and oligonucleotides have been published very recently. These studies, based mainly on NMR spectroscopy, mass spectrometry, and molecular modeling, allow the explanation of the biological activities of the QPA at the molecular level and represent a powerful tool for designing new and more specific substances.

Acknowledgements

The authors would like to thank Dr. Jaromír Marek and Dr. Marek Nečas (Masaryk University, Brno) for their help with compiling the crystallographic data. The financial support of the Ministry of Education of the Czech Republic (MSM0021622413 and LC06030 to R.M.) and the Grant Agency of the Czech Republic (525/04/0017 to L.G.) is gratefully acknowledged.

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