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# THE CHEMICAL AND BIOLOGICAL PROPERTIES OF PROTOPINE AND ALLOCRYPTOPINE

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**Abstract** – The isoquinoline alkaloids, protopine and allocryptopine are components of numerous phytopreparations. The wide spectrum of biological activities reported for these alkaloids include multiple actions on the cardiovascular system, anti-thrombotic, anti-inflammatory, anti-spasmodic, neuroprotective, anti-bacterial, anti-viral, anti-fungal and anti-parasitic activities. This review aims to summarize recent knowledge on the basic chemistry, analysis, above biological activities, and application of both alkaloids published within the period 1995-2010.

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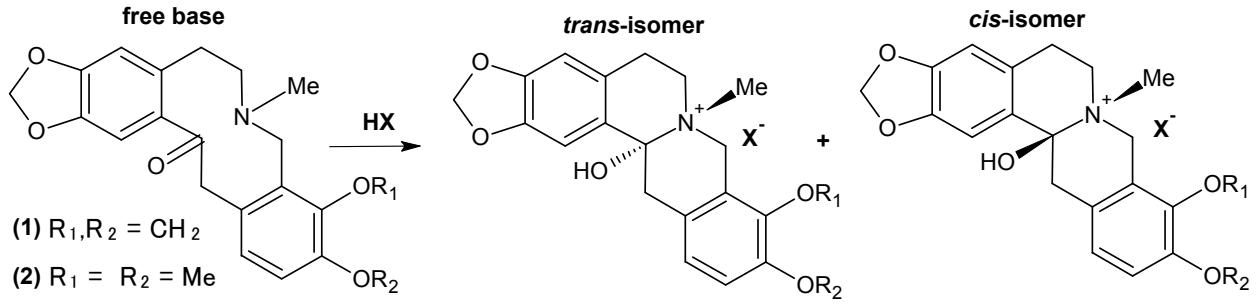
## 1. INTRODUCTION

Protopine and allocryptopine (**1** and **2** in Scheme 1) are isoquinoline alkaloids found primarily in the plant families Fumariaceae, Papaveraceae, Berberidaceae, Ranunculaceae, Rutaceae, and Sapindaceae. The protopine alkaloids are synthesized in plants from ubiquitous L-tyrosine via the (S)-reticulin pathway[1] as protection against biotic stressors. The first finding of protopine (PR) and allocryptopine (AL) in plants was reported by Hesse and Selle at the end of the 19$^{th}$ century.[2,3] However, systematic research on protopine alkaloids began in the sixties of the last century when the structure of the main protopines was clearly identified.[4,5] During the last quarter of the 20$^{th}$ century, new methods for the analysis of both alkaloids in biological material were developed and their biological activities, toxicological parameters and biotransformation pathways were reported.[6] Currently, research on PR and AL is focused on elucidating the molecular biological mechanisms of action, discovery of further biological activities, and their applications as active constituents in human and veterinary phytopreparations. The chemical and selected pharmacological properties of these alkaloids were reviewed by Guinaudeau,[7] Preininger,[6] Onda and Takahashi.[8] Our article is directed to the relevant literature published within the period 1995-2010 on the analysis and mainly biological effects/applications of PR and AL. It is not intended to be more comprehensive.

## 2. CHEMICAL PROPERTIES

PR and AL are usually formulated as free (tricyclic) base with a ten-member heterocyclic ring containing one tertiary nitrogen and carbonyl group stabilized by strong electrostatic interaction. This fundamental structure is typical for all alkaloids of the protopine group (see review[8-10]). However, under acidic conditions PR and AL form tetracyclic salts with quaternary nitrogen.[11] The formation of salts from free bases is connected with transannular interaction between the tertiary nitrogen and the carbonyl group. The PR/AL salt form is a tetracyclic system similar in structure to the protoberberines (a group of isoquinoline alkaloids[12]). The above two structural states of the heterocyclic rings of PR and AL are controlled by the pH of the environment in which they occur. In addition, they can be found as *cis* and *trans* isomers (Scheme 1).[13] These isomers have been recently studied by nuclear magnetic resonance (NMR) spectroscopy and selected stereochemical tools.[14] AL also exists in two interconvertible forms, α- and β-AL. The study of these two forms of AL using X-ray diffraction analysis concluded that α- and β-AL are crystal modifications of AL differing in their crystal packing without substantial distinctions in the conformation of the skeleton.[15] For selected aspects of the chemistry of PR and AL see Table 1. In publications found on the biological effects and analyses of these alkaloids in complex biological matrices, neither *cis*/*trans* isomerisations nor differences between α- and β-AL, were taken into account.




**Scheme 1.** PR **(1)** and AL **(2)** free bases and their tetracyclic salts as *trans* and *cis*-isomers

## 3. ISOLATION AND DETERMINATION

A number of analytical methods and procedures for the identification and quantification of PR and AL in biological samples have been proposed in recent years. The isolation of these alkaloids[2,3] is relatively simple and their synthesis has been described by several laboratories (ref.[16] and Table 1). On this basis, the pure compounds (model standard solutions) are available for confirmation of their identity in biological samples and validation of analytical procedures.[17]

Different approaches have recently been explored for the isolation and extraction of alkaloids, including PR and AL. Usually, the alkaloids from plant dried material are extracted by solvent extraction with methanol and/or ethanol in combination with hydrochloric acid and re-extraction with chloroform.[18,19] For better transition of PR and AL from solid samples to an appropriate organic solvent, the extraction can be improved at higher temperature, in ultrasonic bath (ultrasound-assisted extraction),[17,20] using Soxhlet apparatus,[21] special solid-liquid extractor,[22-24] and/or microwave-assisted extraction procedures.[25] The crude extracts are purified by solid-phase extraction (SPE)[20] based on retention (usually reversed-phase or ion-exchange) of the alkaloids on the SPE sorbent. The retained analytes may be washed, eluted using an elution solvent and than analyzed. SPE has been shown suitable not only for purification of plant extracts but also for simple preparation of urine samples, for example, preparation of urine for GC-MS analysis of PR and its metabolites.[26]

Complete analyses of the isoquinoline alkaloids including the most recent applications in pharmaceutical and biomedical research are reviewed in refs.[27-29] PR and AL have been studied by paper chromatography,[30] thin-layer chromatography,[31] high-performance liquid chromatography (HPLC),[17,18,32] counter-current chromatography,[33] gas chromatography (GC),[21,34] and capillary electrophoresis (CE)[35-37] often in connection with ultraviolet-visible diode-array detection (UV-Vis DAD) systems. The absorption maxima of the alkaloids can be observed around 230 and 280 nm depending on the analytical conditions. From HPLC columns, $C_8$ and $C_{18}$ reversed phases are predominantly used for separation of the alkaloids.[17,18,38] The $C_{18}$ reversed-phase HPLC columns with different separation parameters were tested

for analysis of PR, AL and other main alkaloids in *Macleaya cordata* methanolic extracts. In addition, optimized HPLC were used to study the distribution of the alkaloids in extracts from *M. cordata* roots.[17] Isocratic[18] and linear gradient[17,39,40] elution with mobile phases consisting of acetonitrile with acidified aqueous-based solvents enabled optimal resolution of chromatographic separations. The limit of detection of HPLC-UV-Vis DAD varied in ng of the alkaloids per g of sample.[17,39]

**Table 1.** Chemical properties and basic information on PR and AL

| | **PROTOPINE** | **ALLOCRYPTOPINE** |
|---|---|---|
| Systematic name | 4,6,7,14-Tetrahydro-5-methylbis[1,3]-benzodioxolo[4,5-*c*:5',6'-*g*]azecin-13(5*H*)-one); $C_{20}H_{19}NO_5$, ref.[41] | (5,7,8,15-Tetrahydro-3,4-dimethoxy-6-methylbenzo, [1,3] dioxolo[4,5-*k*] [3]benzazecin-14(6*H*)-one); $C_{21}H_{23}NO_5$, ref.[41] |
| Main plant sources | Papaveraceae (*Argemone, Bocconia, Eschscholtzia, Glaucium, Macleaya, Sanguinaria, Chelidonium, Papaver, Hylomecon, Stylomecon*), Fumariaceae (*Corydalis, Dactylicapnos*), Ranunculaceae (*Thalictrum*), Rutaceae (*Fagara*), *Berberidaceae* (Berberis), Sapindaceae (*Pteridophyllum*)[42] | |
| Isolation, chemical structures and properties | First isolation,[2] structure,[43] X-ray crystallography,[5] NMR,[4,14] UV spectrometry,[44] mass spectrometry,[45] $M_W$ 353.374 | First isolation,[3] structure,[46] X-ray crystallography (α- and β-form[15]), NMR,[47] UV spectrometry,[44] mass spectrometry,[48] $M_W$ 369.416 |
| Biosynthesis | Enzymatically from the primary metabolite, L-tyrosine, via (S)-coclaurine, (S)-retikulin, (S)-scoulerine, and (S)-stylopine intermediates; reviewed in ref.[1] | |
| Organic synthesis | First organic synthesis, Haworth and Perkin[49]; Bentley's method, Hanaoka's method, Rönsch's method, reviewed in ref.[16] | |

Identification of the alkaloids in biological samples and their structural analysis can be carried out using mass spectrometry (MS) and $^1H/^{13}C$ NMR spectroscopy.[20] Electrospray ionization (ESI), operating in positive mode, has been shown suitable for analysis of PR and AL based on chromatographic/electrophoretic-MS interfaces. MS analyzers have been used in different configurations such as single quadrupole,[40] ion trap,[36,37] Fourier transform ion cyclotron resonance,[19] and tandem MS (MS/MS).[18,19,39] The alkaloids are identified by their molecular ions (*m*/*z* 354 for PR and *m*/*z* 370 for AL) and specific fragmentation products; the fragmentation pathways for PR and other alkaloids are described in ref.[39]

Using these analytical methods, PR and AL have been determined in the plants, *Eschscholtzia californica,*[18] *Macleaya cordata,*[17] *Fumaria* ssp.,[21] *Corydalis* spp.,[20,50] and biosynthesis of the alkaloids was confirmed in *Papaver somniferum.*[19] In general, the content of the alkaloids varied in the herbal material depending on

the conditions of plant growth[51] and the quantification data varied in relation to the isolation procedure used. Usually, micrograms and/or milligrams of the PR and AL per gram of plant tissue are found. For example, the roots of *M. cordata* contained around 6.7 PR/13.1 AL mg/g of dry weight.[17]

### 3.1. Analysis of protopine and allocryptopine in clinical samples

Clinical material as urine[26,34] and plasma[32,40] have been examined. PR biotransformation has been investigated in rat, horse, and human urine where metabolites, excreted as conjugates, were confirmed using positive ESI with electron impact MS.[34] The ESI MS method in combination with HPLC was applied in monitoring PR in rat plasma after oral administration of phytopreparations from *Corydalis decumbens*.[40] A complete separation and quantification of nanogram quantities of PR, AL, and the whole spectrum of their metabolites[34,40,52] in real samples is now possible using modern HPLC and MS methods. This fact is a key parameter in the search for new PR and AL derivatives, products of biotransformation and for quality control of phytopharmaceutical products containing them.

## 4. BIOLOGICAL PROPERTIES

### 4.1. Effects on cardiovascular system and relaxant effects on smooth muscle

PR has been found to have multiple effects on the cardiovascular system, including anti-arrhythmic, anti-hypertensive, negative inotropic and vasodilator effects.[6,8] Anti-arrhythmic effects have been also demonstrated for AL. The mechanisms of these activities at cellular and molecular levels have been recently further explored.

The major PR actions on the heart are explained as relating to its electrophysiological properties, namely its effects on action potentials and various types of ionic currents. In single isolated ventricular myocytes from guinea-pig, extracellular application of PR markedly and reversibly shortened the action potential duration, and decreased the rate of upstroke, amplitude and overshoot of the action potential in a dose-dependent manner. Additionally, it produced slight but significant hyperpolarization of the resting membrane potential. Detailed analysis of its effect on channel currents showed that PR is a multiple-channel blocker suppressing both L-type $Ca^{2+}$ channel and the inward rectifier potassium channel, delayed rectifier potassium and sodium channels. It was concluded that PR is not a selective $Ca^{2+}$ channel antagonist as previously suggested; rather it acts as a promiscuous cation channel inhibitor.[53] The effect of PR on the ATP-sensitive $K_{ATP}$ channel and large conductance $Ca^{2+}$-activated $BK_{Ca}$ channel expressed in human embryonic kidney cells (HEK-293) was investigated.[54] PR concentration-dependently inhibited $K_{ATP}$ channel currents by targeting the SUR1 subunit. On the other hand, $BK_{Ca}$ channels were reported not to be modulated by PR.[54] AL produced a blocking effect on the transient outward potassium current I(to) in cardiac myocytes and this may be an important mechanism in

its anti-arrhythmic effect. In a rabbit left-ventricular myocytes model, AL decreased the amplitude and density of the I(to) in a concentration-dependent and frequency- or use-independent manner, and decreased the transmural gradient of the I(to).[55]

The vasodilator effects of PR examined in isolated rabbit aorta were found to be related to elevation of cAMP and cGMP. In rat aortic strips, PR dose-dependently inhibited isotonic contractions induced by noradrenaline and high potassium levels. PR (50 and 100 μM) had no effect on resting intracellular free $Ca^{2+}$ concentration ($[Ca^{2+}]$) in vascular smooth muscle cells of rat aorta but significantly decreased $[Ca^{2+}]$ elevated by noradrenaline and high $[K^+]$. In the presence of noradrenaline, PR also affected activities of the membrane and cytosolic protein kinase C (PKC) in the aortic strips indicating PR promotion of the PKC translocation from cytosol to cell membrane. Thus, it was concluded that the PR vasodilative effect may be the comprehensive result of its decreasing effect on cytosolic $Ca^{2+}$ and increasing effect on cAMP and cGMP, as well as its influence on the PKC.[56]

PR further displays anti-thrombotic and anti-inflammatory activities associated with its effects on intracellular $Ca^{2+}$ concentration, potent inhibition of platelet-activating factor (PAF), and thromboxane synthesis. In a study of the PR influence on cytoplasmic $Ca^{2+}$ concentration in rabbit platelets it was found that PR inhibited not only $Ca^{2+}$ release but also $Ca^{2+}$ influx.[57] PR decreased ADP-, arachidonic acid (AA)-, and PAF-induced $Ca^{2+}$ influx[58] and exhibited significant inhibitory activity towards ADP-, AA-, collagen-, and/or PAF-induced platelet aggregation. The $IC_{50}$ values were much less than those observed for acetylsalicylic acid. PR selectively inhibited the synthesis of thromboxane A2 via the cyclooxygenase pathway but had no effect on the lipoxygenase pathway in platelets.[59] *In vivo*, pretreatment with PR protected rabbits from the lethal effects of AA and PAF in a dose-dependent manner. PR also inhibited carrageenan-induced rat paw oedema with a potency three-fold higher than acetylsalicylic acid.[59]

Both alkaloids possess anti-spasmodic and relaxant effects on smooth muscle. Mild anti-spasmodic and relaxant activity was observed in different anti-spasmodic test models on isolated ileum of guinea-pigs. PR exhibited the known papaverine-like musculotropic action and antagonized carbachol and the electric field stimulated contractions.[60] It also reduced morphine withdrawal in guinea-pig ileum and this finding has raised the possibility of PR in the treatment of drug abuse.[61] AL caused a concentration-dependent contraction of rat isolated urinary bladder and relaxation of rat ileal smooth muscle. The effects of AL in the presence of the inhibitors of phosphodiesterase and soluble guanylate cyclase, and α-adrenergic receptor blockers were investigated. The results suggest that AL induces a relaxing effect on the ileum by inhibiting phosphodiesterase and has contractile effects on the urinary bladder by affecting the α-adrenergic receptors in this tissue.[62]

### 4.2. Anti-oxidative and neuroprotective properties

Several studies reported neuroprotective effects for both alkaloids. In the search for novel drug candidates for the treatment of Alzheimer's disease (AD), PR and AL were identified as potent acetylcholinesterase inhibitors both *in vitro* and *in vivo*. PR displayed significant inhibitory activity among the alkaloids extracted from *Corydalis speciosa* and *Corydalis ternata.* The anti-acetylcholinesterase activity of PR was dose-dependent, specific, reversible and competitive in manner.[63,64] Alkaloid extracts from aerial parts of 20 species of the genus *Fumaria* (PR and AL are constituents) were screened for their inhibitory effect on acetylcholinesterase activity by Ellman's method.[65] While galanthamine ($IC_{50}$ = 5.8 μM), the standard drug for AD, showed 49 % inhibitory activity, all of the extracts had higher activity than galanthamine, ranging from 85 to 97 %. Among the alkaloids obtained from the extract of *Fumaria vaillanti*, the most active species in this assay, PR ($IC_{50}$ = 1.8 μM) and AL ($IC_{50}$ = 1.3 μM) were the most potent inhibitors.[65] PR was shown to modulate glutamate metabolism in the brain through activation of glutamate dehydrogenase (GDH). PR and alkalized extract of the tuber of *Corydalis ternata* (PR is a main component) decreased glutamate levels and increased the GDH activity in rat brains after treatment. PR reduced glutamate levels up to 23 %, and increased GDH activity 1.6-fold compared to control values. When stimulatory effects on GDH activity were examined *in vitro* with two types of human isoenzymes, hGDH1 (house-keeping GDH) and hGDH2 (nerve-specific GDH), it was found that nerve-specific GDH was more sensitively affected.[66] Glutamate excitotoxicity and calcium overload are both involved in the pathophysiological sequelae of stroke and PR in this regard offers promise as a protection against these pathologies. Further, the neuroprotective effects of PR on foecal cerebral ischeamic injury were shown in rats. Pre-treatment with PR reduced the cerebral infarction ratio and serum lactate dehydrogenase activity, and improved the ischemia-induced neurological deficit score and histological changes of brain in a dose-dependent manner. It also increased superoxide dismutase activity in serum indicating that the neuroprotective effect of PR is partially related to its antioxidant properties. Further, PR decreased total calcium and significantly reduced apoptosis detected as number of TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling)-positive cells in the ischemic brain tissue.[67] The role of anti-oxidant and anti-apoptotic mechanisms in the neuroprotective action of PR was further supported by investigation of PR effects on acute oxidative injury in PC12 cells, on an *in vitro* model system extensively used to study neuronal differentiation and survival. Pretreatment of PC12 cells with PR improved cell viability, enhanced superoxide dismutase, glutathione peroxidase and catalase activity, and decreased lipid peroxidation, in $H_2O_2$ injured cells. PR also reversed increased intracellular [$Ca^{2+}$], reduced mitochondrial membrane potential, caused by $H_2O_2$ injury, inhibited $H_2O_2$ induced caspase-3 expression and cell apoptosis. These results confirm that PR is capable of relieving oxidative stress and apoptosis, at least in part, by antioxidant mechanisms and $Ca^{2+}$ antagonism.[68] Häberlein *et al*.[69] studied the

effect of an extract of *Chelidonii* herba on the gamma-aminobutyric acid ($GABA_A$) receptor, a modulator of many physiological functions in the central nervous system and a target for a variety of drugs used in the treatment of neurological and psychiatric disorders. *In vitro* binding studies clearly indicate that PR is responsible for the positive synergistic effect of *Chelidonii* herba extract and the allosteric modulation of the $GABA_A$ receptor. The simultaneous presence of small amounts of AL and stylopine elevated the action of PR. As PR, AL and stylopine had no influence on the specific binding of [$^3$H]flunitrazepam, it was suggested that the positive cooperative modulation of the $GABA_A$ receptor was not based on interaction of these alkaloids with the benzodiazepine binding site. Radioreceptor assays rather provided evidence for the interaction of these alkaloids with the chloride channel of the $GABA_A$ receptor. PR was identified as an inhibitor of both serotonin transporter (SERT) and noradrenalin transporter (NERT) in *in vitro* assays.[70] Considering that NERT and SERT are the cellular targets for most clinically used anti-depressants, the anti-depressant-like effect of PR was also assessed. 5-Hydroxy-DL-tryptophan(5-HTP)-induced head twitch response (HTR) and tail suspension test were adopted to study whether PR has anti-depression effects in mice. In the HTR test, PR dose dependently increased the number of 5-HTP-induced HTR. It also produced a dose-dependent reduction in immobility in the tail suspension test.[70] When mice were pretreated with PR, the alkaloid significantly reduced scopolamine-induced memory impairment. In fact, PR had an efficacy almost identical to that of velnacrine, a tacrine derivative developed by a major drug manufacturer to treat AD, at an identical therapeutic concentration. The authors suggested new possibilities for the use of PR in the treatment of mood disorders, such as mild and moderate states of depression, as well as in alleviating the memory impairments of the dementias and AD.[64]

### 4.3. Hepatoprotective effects

The hepatoprotective potential of PR was demonstrated in D-galactosamine induced hepatotoxicity in rats. PR decreased serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubine, increased levels of reduced glutathione and lowered lipid peroxidation. PR in doses of 10-20 mg *per os* was found to be as effective as the standard drug silymarine.[71] Pretreatment of rats with PR also significantly reduced AST and ALT levels in paracetamol and $CCl_4$ induced hepatic damage.[72]

### 4.4. Anti-bacterial, anti-viral, and anti-parasitic activities

Both PR and AL were screened for anti-bacterial, anti-viral, anti-fungal and anti-parasitic effects. Anti-bacterial and anti-viral activities of PR were found for a number of bacterial strains including *Helicobacter pylori*[73] and RNA Parainfluenza (PI-3) virus.[74] PR and AL were identified as the

compounds responsible for the significant anti-fungal activity of the plant *Glaucium oxylobum* against *Microsporum gypseum*, *Microsporum canis*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum*.[75] In *in vitro* anti-malarial assay, PR displayed promising anti-plasmodial activity against the malaria protozoan *Plasmodium falciparum*, both wild type (TM4) and multi-drug resistant (K1) strains with $IC_{50}$ value 1.5 μg/mL.[76] PR also exhibited strong nematocidal activity and was proposed as a potential treatment for strongyloidosis.[77] Among the alkaloids tested to find new anthelmintics against parasites living in host tissues, AL showed significant nematocidal activity against the larva of dog roundworm, *Toxocara canis* and low cytotoxicity. Thus AL was proposed as a potentially effective anthelmintic.[78] PR in combination with ENT 8184 or piperonyl butoxide caused a significant reduction in the fecundity, hatchability and survival of young snails *Lymnaea acuminate*.[79] Exposure to the molluscidal component of *Argemone mexicana* (PR and sanguinarine) exhibited a significant decrease in the levels of protein, free amino acid, DNA and RNA, reduction in phospholipid levels and a simultaneous increase in the rate of lipid peroxidation in the nervous tissue of treated snails.[80]

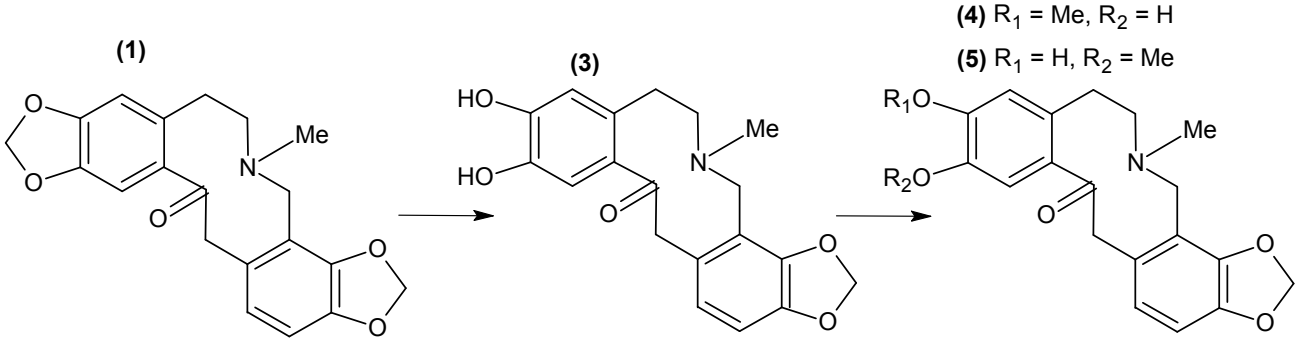
### 4.5. Cytotoxic and anti-proliferative activities

The cytotoxic and anti-proliferative activities of PR on tumour (LSCC-SF-Mc29, LSR-SF-SR) and nontumour (L929, MDBK) cells were evaluated. PR expressed various degrees of cytotoxicity and anti-proliferative activity against tested tumour cells but was less effective than the anti-tumour drugs oxaliplatine, vinblastine and cyclophosphamide. PR was much less toxic and cytostatic for nontumour cells from L929 and MDBK lines. The compound inhibited the colony-forming ability of tumor cells in a dose dependent manner and in doses >0.001 μM blocked the colony-forming capacity of normal murine bone-marrow cells.[81,82]

## 5. PHARMACOLOGY

Few studies have focused on the metabolism and pharmacokinetics of PR. Studies on PR biotransformation have revealed that in rats, PR undergoes only extensive demethylenation of the 2,3-methylenedioxy group followed by catechol-*O*-methylation (Scheme 2, **3**-**5**). All phenolic hydroxy metabolites identified in rat urine were found to be partly conjugated with glucuronate and/or sulfate.[34] In the horse and human, PR was almost completely metabolized with little of the unchanged parent compounds excreted in the urine. The metabolite pool was qualitatively similar in both species and included the metabolites **3-5** described above. In addition, three tetrahydroprotoberberine metabolites formed by closure of the bridge across N-7 and C-14 were identified. At low PR doses (0.04 mg/kg body weight) tetrahydroprotoberberine metabolites were found to be the major urinary metabolites in humans, at higher doses (3.5 mg/kg body weight) a significant shift in PR metabolite distribution in favour of

metabolites **3-5** was observed.[26] The role of the cytochrome P450 isoenzymes (CYP) in the metabolism of *Eschscholtzia californica* alkaloids californine and PR was investigated using rat liver microsomes. Mainly CYP2D1 and CYP2C11 were involved in PR demethylenation, while CYP1A2 and CYP3A2 showed only minor contribution.[52] The pharmacokinetics of PR was investigated in rats after intravenous administration of 10 mg/kg body weight. The concentration-time curve of PR in rat corresponded to a two-compartment open model, for details see refs.[40,83] The time to reach the maximum plasma concentration in rats after oral administration of PR containing *Rhizoma Corydalis Decumbentis* extract (2 g/kg body weight, content of PR 15.44 mg/kg) was 3.50 ± 0.55 h and the elimination half-time was 4.98 ± 1.64 h.[40,83]




**Scheme 2.** Metabolization of PR, metabolites **3-5** after deconjugation in rat,[34] horse and human[26]

## 6. PHYTOPREPARATIONS

PR and AL are believed to be active constituents of many medicinal plants, *e.g. Chelidonium, Fumaria*, *Macleaya*, *Sanguinaria* spp., used in veterinary and human phytotherapeutics (Table 2). Both alkaloids are present in Sangrovit, a natural feed additive improves feed uptake in animals (appetite stimulation).[84-86] The active component of Sangrovit is *Macleaya cordata* herb containing a mixture of protopine and benzo[*c*]phenanthridine alkaloids, ~20 mg PR and AL/g of Sangrovit. Herbiplant CS, Neoplasene and Xxtera are other protopine containing preparations. Herbiplant CS is an appetite stimulant prepared from *Chelidonium majus* with beneficial influence on digestibility and nutrient uptake in animals.[87,88] Neoplasene and Xxtera are preparations from *Sanguinaria canadensis* which can be used as an alternative treatment for various types of skin lesions, primarily sarcoidosis in farm animals.[89,90] For treatment of dermatological conditions, Fumitory, prepared from *Fumaria officinalis* herb and/or its extracts, can also be used. This phytopreparation showed positive effects against eczema.[91,92] In the recent literature, there is interest in the phytopreparation Iberogast (also known as STW 5) consisting of extracts from *Chelidonium majus* and other herbs. Iberogast is a gastro-intestinal phytoterapeutic medication against non-ulcer dyspepsia and irritable bowel syndrome.[93,94] Protopine alkaloids are also present in the hepatoprotective preparation Hepabene which is based on *F. officinalis* and *Silybum marianum* extracts.[95]

Finally, PR is a component of the anti-cancer drug Ukrain. Major components of Ukrain are *Chelidonium majus* alkaloids, including protopines, treated with the alkylating agent thiotepa (N,N'N'-triethylenethiophosphoramide). Ukrain has been demonstrated to possess anti-neoplastic and immunomodulatory properties. It inhibits the growth of cancer cell lines *in vitro*, tumor mass reductions *in vivo*, and partial and complete remissions in cancer patients.[96]

**Table 2.** Selected phytopreparations containing PR and/or AL used in veterinary and human medicine

| Phytopreparation | Description/Application | Active compounds | Ref. |
|---|---|---|---|
| *PR/AL is one's of active components* | | | |
| Fumitory | *Herba fumariae* or its extract used as a laxative, diuretic, and as a treatment for dermatologic conditions, such as eczema. | The principal bioactive constituents of *Fumaria officinalis* is PR, fumaricine, fumariline, and indenobenzazepines, and bioactive phenolics. | 91,92 |
| Hepabene | Hepatoprotective preparation based on *Fumaria officinalis* and *Silybum marianum* extracts. | *F. officinalis* constituents (see above) and flavonolignans, especially silybin. | 95 |
| Herbiplant CS | Productivity stimulant and preparation with beneficial influence on a digestibility in animals, prepared from *Chelidonium majus*. | Isoquinoline alkaloids from *C. majus* (sanguinarine, chelerythrine, chelidonine, homochelidonine, PR, AL, berberine, coptisine, etc.). | 87,88 |
| Sangrovit | Appetite stimulant and antibacterial protective feed additive based on *Macleaya cordata* powdered material. | Alkaloids from *M. cordata* (sanguinarine, chelerythrine, PR, AL, and other). | 97 |
| *PR/AL is trace component* | | | |
| Iberogast | Gastrointenstinal phytotherapeutic medication consisted of nine extracts from different plants. | Complex mixture of constituents containing *Chelidonium majus* alkaloids. | 93,94 |
| Ukrain | Preparation for malignant neoplasm treatment based on *Chelidonium majus* alkaloids. | Main constituents from *C. majus*, mixture of chelidonine and protopine type of alkaloids, treated with alkylating agent thiotepa. | 96 |
| Neoplasene<br>Xxtera | Preparations from *Sanguinaria canadensis* alkaloids used to treat various types of skin lesions, including sarcoidosis. | Mixtures of *S. canadensis* alkaloids, mainly sanguinarine, chelerythrine, PR, and AL. | 89<br>90 |

Of patented preparations and procedures, AL is a component of therapeutic mixtures for the treatment of HIV-1 and HIV-2. The mixture, consisting of AL, nimodipine, potassium iodide, potassium iodate, inuline, silver, zinc, chromium, orotic acid and desferrin, has particular application in the treatment of the AIDS. The beneficial effect is due to blocking attachment of the gp 160 protein of the HIV virus capsule to the receptors of the $CD4^+$ cells of the human immune system, as well as blocking the intracellular processes related to HIV-1 and HIV-2 virus replication. The weight ratio of all of these components can vary as described in the patent WO/2004/066954. It is important to note that the anti-HIV effects of protopines have not been proven scientifically.

## 7. CONCLUSION

This review presents data on the basic chemistry, analytical methods and wide spectrum of biological activities of the protopine alkaloids, PR and AL including: multiple actions on the cardiovascular system, anti-thrombotic and anti-inflammatory activities, anti-spasmodic, relaxant effects on smooth muscle, neuroprotection, anti-bacterial, anti-viral, anti-fungal and anti-parasitic activities. The mechanisms of their action are shown to involve interaction with cell membrane calcium channels, resulting in changes in intracellular ion concentration, inhibition of platelet aggregating factor and thromboxane synthesis, allosteric modulation of $GABA_A$ receptor, inhibition of serotonin and noradrenalin transporters, inhibition of acetylcholinesterase, glutamate dehydrogenase, phosphodiesterase, and suppression of oxidative stress and apoptosis. The therapeutic use of PR in the treatment of some cardiovascular, nervous system and psychiatric disorders has been suggested. Finally a possible preparation for the treatment of HIV-1 and HIV-2 viruses containing pharmaceutically effective amounts of AL has been patented.

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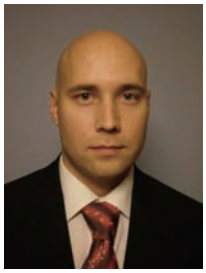

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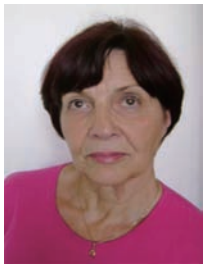

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