Investigations of diterpene alkaloids isolated from *Aconitum anthora* L. and *A. moldavicum* L., and of aconitine-derived lipo-alkaloids

Summary of Ph.D. Thesis

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1. INTRODUCTION

The buttercup family (*Ranunculaceae*) has more than 2000 species and by producing specific compounds possessing a very wide range of pharmacological characteristics and physiological effects, thus it has been in the focus of an ever-growing scientific interest worldwide. Species belonging to the most actively investigated genera (*Aconitum*, *Delphinium*, *Consolida*) are widely distributed throughout the Northern hemisphere. Based on common empiric knowledge these plants are considered to be poisonous. Species of these genera accumulate highly toxic diterpene alkaloids (DAs), which have attracted considerable interest motivated by their complex structures, interesting chemistry and noteworthy physiological effects.

A widely distributed species, *A. napellus*, gained certain healing reputation at the beginning of the European professional official therapy as its roots and main alkaloid, aconitine, were used for the treatment of trigeminal neuralgia. From the beginning to the late second third of the 20th century, in parallel with the recognition of the very narrow therapeutic range of DAs, the medicinal use of aconite drugs had disappeared from Western medicinal practices. Nevertheless, in Far Eastern traditional medicinal systems aconite drugs have been applied for centuries as painkillers and antirheumatic agents. Several unprocessed and processed aconite drugs are still official in the national pharmacopoeias of numerous Far Eastern countries.

In spite of our rapidly growing knowledge concerning the chemistry of processed drugs there is still no sufficient information on their pharmacology and toxicology. With the worldwide increasing spread of these medicinal systems, and with the increasing public interest towards phytomedicines, phytoanalytical studies on aconite drugs are of growing importance with respect to their safe application. In consequence of the harmonization of Traditional Chinese Medicine (TCM) and modern, evidence based medicinal systems aconite preparations are attracting increasing interest today in Western medicine too. The incorporation of *Aconitum* drugs into the European Pharmacopoeia is currently in progress.

Nowadays special emphasis has been placed to research activities aiming the study of the effects of traditional processing methods on the chemical composition of aconite drugs, elaboration of adequate analytical methods for the quality control of these processing methods, and pharmacological-toxicological evaluations of their alkaloids.

2. AIMS OF THE STUDY

In 2001, the research group of HOHMANN *et al.* (Department of Pharmacognosy, University of Szeged) has started a research programme dealing with *Aconitum* species native to the Carpathian Basin. The aim of this programme is to study these species since chemical and pharmacological characteristics of the rare and/or endemic *Aconitum* species are partially or completely unexplored. By joining to this ongoing comprehensive research the main goals of my work were to

1. provide review of recent advances in the topic of diterpene alkaloids,
2. phytochemically examine the alkaloid contents of *A. anthora* and *A. moldavicum*, i.e. isolate their DAs via preparative chromatographic methods in order to gain information concerning the chemistry of the species of the Ranunculaceae family,
3. elucidate the structures of isolated compounds via NMR and HRMS techniques, provide characteristic spectral data on the isolated new compounds, and supplement missing NMR data on the already-known compounds,
4. carry out the semisynthesis and purification of a series of lipo-alkaloids (LAs) derived from aconitine,
5. in the frame of co-operations, carry out and evaluate *in vitro* anti-inflammatory activities of LAs and hERG and $Na_v1.2$ channel activities of previously or newly isolated DAs,
6. carry out quantitative determination of toxic diester diterpene alkaloids (DDAs) in authentic processed *A. carmichaelii* and *A. kusnezoffii* samples.

3. MATERIALS AND METHODS

Plant material of *A. anthora* was collected at Füzéri Várhegy and Tar-kő in the North-Hungarian Mountains in the flowering period in September 2002, and of *A. moldavicum* was collected near to Eger in October 2006.

For the isolation of DAs several chromatographic methods (open-column chromatography (CC), centrifugal planar chromatography (CPC), gel-filtration chromatography (GFC), preparative layer chromatography (PLC), thin-layer chromatography (TLC), vacuum liquid chromatography (VLC)) were applied in a consequent and combined manner. Chromatographic fractions were monitored by TLC on Al_2O_3 plates, and visualized by spraying with *cc*. H_2SO_4, followed by heating or with Dragendorff's reagent.

Structure elucidation was carried out by means of HRESIMS and extensive 1D and 2D NMR spectroscopic methods (^{1}H-^{1}H COSY, NOESY, HSQC and HMBC experiments).

As for the semisynthesis of LAs all chemical substances used for the reactions were purchased as highly purified test reagents. In the transesterification reactions the reaction mixtures were heated in an oil bath for 3 h under vacuum (10 mbar). In the reactions 20 mg aconitine was esterified by 40 mg lauric, myristic, stearic, palmitoleic, oleic, α- and γ-linolenic, eicosanoic, 11*Z*-eicosenoic, 11*Z*,14*Z*-eicosadienoic, 8*Z*,11*Z*,14*Z*-eicosatrienoic, eicosapentaenoic acids, and 25 mg docosahexaenoic acid, respectively. The resulting LAs were purified by multi-step chromatographic methods. The *in vitro* anti-inflammatory activities of the semisynthetically produced LAs were evaluated by COX-1, COX-2 and LTB_4 formation inhibitory assays, in which indomethacine (COX-1, IC_{50} 0.9 μM) and NSB-398 (COX-2, IC_{50} 2.6 μM) were used as positive controls for COX-1 and COX-2, respectively. In the LTB_4 formation inhibition assay zileuton (IC_{50} 5.0 μM) was used as positive control.

For the bioassays on the hERG and $Na_v1.2$ channels a series of previously isolated or semisynthetised alkaloids was selected, including some of those isolated from *A. anthora* and *A. moldavicum*. In the hERG bioassay haloperidol was used as a positive control. Analysis of the effects of DAs on the hERG and $Na_v1.2$ channels were carried out by using the automated whole-cell patch clamp technique, using the QPatch-16 system and CHO cells that stably express the transcript of hERG and human $Na_v1.2$ sodium channels.

For the determination of toxic alkaloid contents, alkaloid titration and HPLC quantification were carried out. For analysis, processed Radix aconiti samples (3 samples of Zhicaowu – Aconiti kusnezoffi praeparata; 2 samples of Zhichuanwu (Shanxi) – Aconiti praeparata (radix); 2 samples of Aconiti carmichaelii radix praeparata/Aconiti radix praeparata; 5 samples of Aconiti radix praeparata/Aconiti radix lateralis praeparata from the Shanghai market; 4 samples of the same drug but from the German market) were obtained from different suppliers. Unprocessed *A. carmichaelii* roots (one sample) were obtained from a pharmacy in China.

In the HPLC analysis the peaks of mesaconitine, aconitine, and hypaconitine were identified by comparison of the HPLC-DAD chromatograms of the extracts of aconite roots with those of the reference solutions. Alkaloid content was calculated by comparison of the sum of the areas under curves (AUC) of mesaconitine, aconitine, and hypaconitine on the basis of the calibration curve established for aconitine. Calibration was established for aconitine based on five concentrations (with a range of 0.05–1.625 μg). Alkaloid titration for the comparison of the measurement results was carried out according the method of the *German Homeopathic Pharmacopoeia*.

4. MAIN RESULTS OF THE STUDY AND THEIR EVALUATION

***Isolation of alkaloids from* A. anthora**

In the case of this species, a classical alkaloid isolation methodology based on solvent-solvent partitioning was applied (**Figure 1**). This method aims at the separation of alkaloids from neutral compounds. The dry herbal sample was ground and percolated with MeOH. After evaporation *in vacuo*, the concentrated extract was diluted with water, and acidified with 4% H_2SO_4. After the removal of neutral materials with $CHCl_3$, the acidic solution was adjusted to pH 9.0 with 5% NaOH, and extracted with $CHCl_3$ to yield the crude alkaloid fraction. This crude fraction was separated by selective multistep chromatographic methods (VLC, PLC, GFC) with the use of Al_2O_3 and Sephadex® LH-20 as stationary phases and different solvent systems yielding 3 pure alkaloids (**ANT–1-3**).

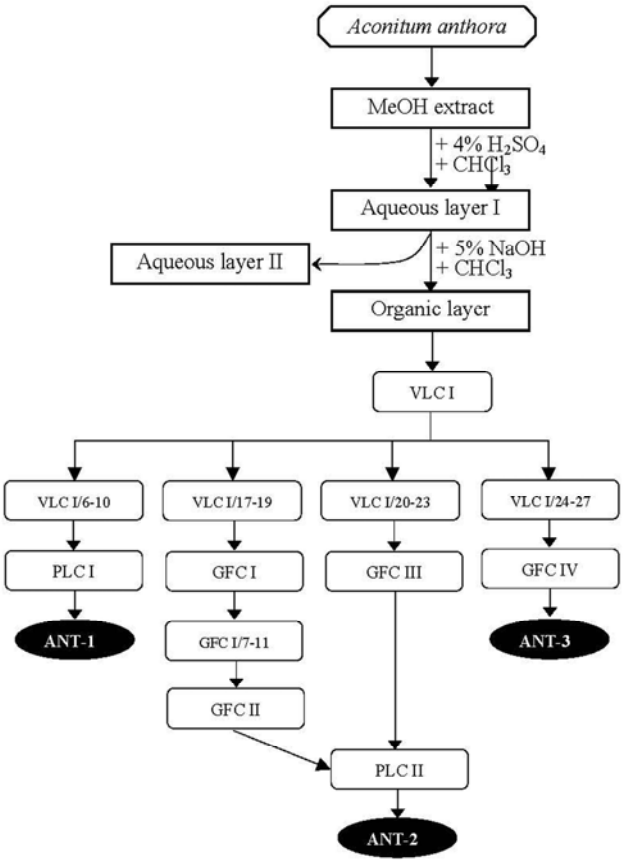


Figure 1. Isolation of alkaloids from *A. anthora*

***Isolation of alkaloids from* A. moldavicum**

In the case of *A. moldavicum*, an isolation procedure in neutral medium was proposed to obtain the alkaloids (**Figure 2**). Reasons for the choice of this method were the facts that the extract of the roots did not contain chlorophyll; moreover, in neutral medium the risk of acidic or alkaline hydrolysis can be minimized. Initially, CC using an Al_2O_3 stationary phase was applied to remove polyphenolic compounds. After extensive chromatographic purification (including CC, VLC, GFC, PLC and CPC) with the use of Al_2O_3 and Sephadex® LH-20 as stationary phases and different solvent systems, 8 pure alkaloids were isolated (**AMO–1-8**).

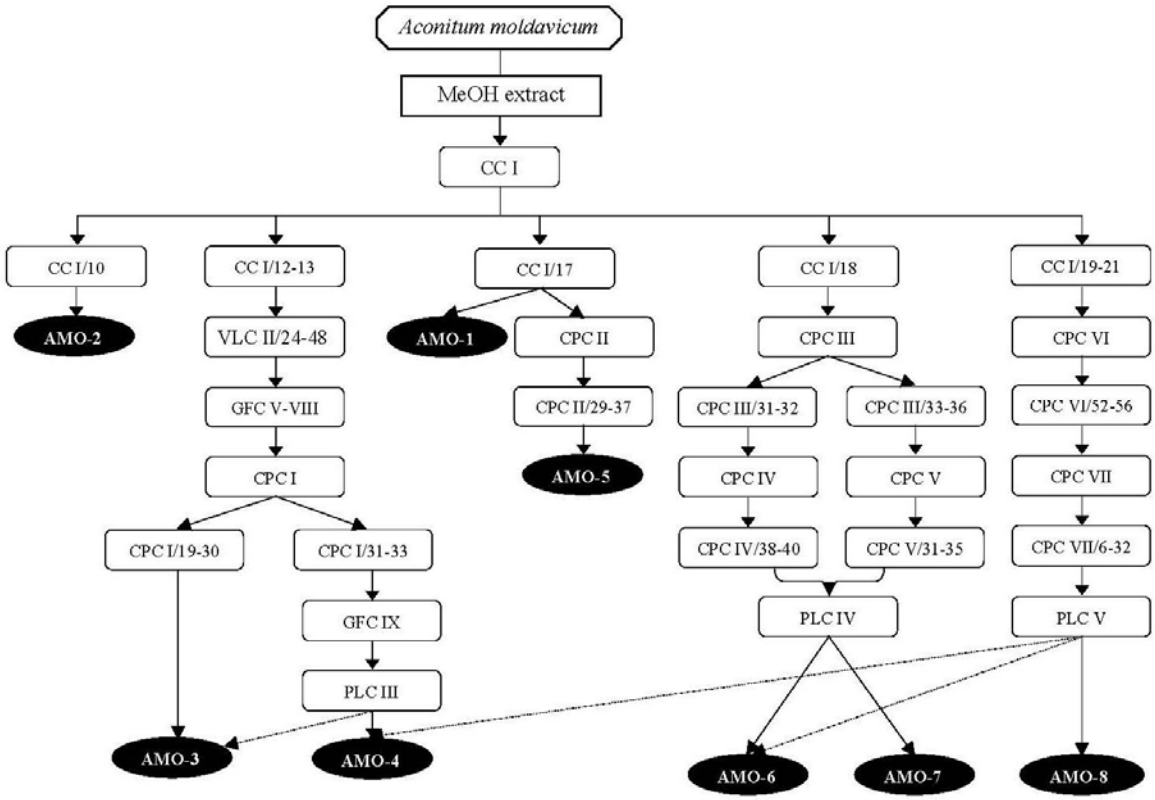


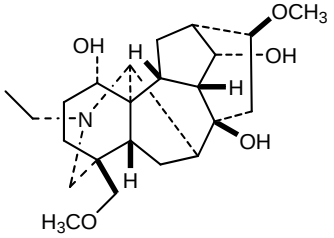
Figure 2. Isolation of alkaloids from *A. moldavicum*

Structure elucidation of isolated compounds

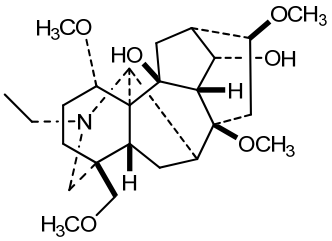
From *A. anthora*, 1 hetisane-type C_{20} [hetisinone (**3, ANT-3**)] and 2 aconitane-type C_{19} DAs [isotalatizidine (**1**, **ANT-1**) and 10-hydroxy-8-*O*-methyltalatizamine (**2**, **ANT-2**)] were identified. The structure and relative configuration of the new alkaloid, 10-hydroxy-8-*O*-methyltalatizamine (**2**, **ANT-2**) were elucidated. ^{1}H- and ^{13}C-NMR chemical shift assignments were determined for this compound for the first time, with a corrected or supplemented assignment in the case of isotalatizidine (**1**, **ANT-1**). Hetisinone (**3**, **ANT-3**) was identified on the basis of the good agreement of measured and previously reported NMR data.

From *A. moldavicum*, 8 aconitane-type C_{19} DAs [delcosine (**4**, **AMO-1**), ajacine (**5**, **AMO-2**), lycoctonine (**6**, **AMO-3**), swatinine (**7**, **AMO-4**), gigactonine (**8**, **AMO-5**), cammaconine (**9**, **AMO-6**), columbianine (**10**, **AMO-7**)], and 1-*O*-demethylswatinine (**11**, **AMO-8**)] were identified. The complete structure and relative stereochemistry of the new alkaloid, 1-*O*-demethylswatinine (**11**, **AMO-8**) were determined. For ajacine (**5**, **AMO-2**), and swatinine (**7**, **AMO-4**), complete ^{1}H chemical shift assignments were also carried out. All other alkaloids, namely delcosine (**4**, **AMO-1**), lycoctonine (**6**, **AMO-3**) gigactonine (**8**, **AMO-5**), cammaconine (**9**, **AMO-6**), and columbianine (**10**, **AMO-7**) were identified on the basis of the comparison of the measured and literature NMR data.

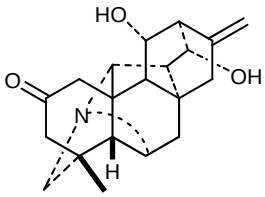
Alkaloids isolated from *A. anthora*



1 (**ANT-1**)

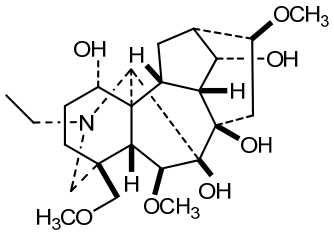


2 (**ANT-2**)

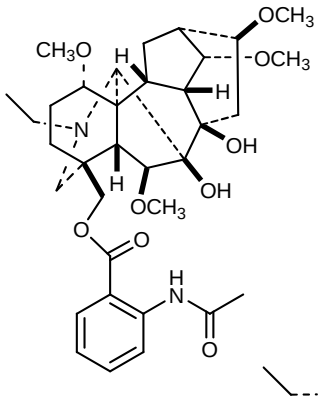


3 (**ANT-3**)

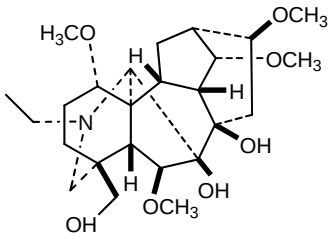
Alkaloids isolated from *A. moldavicum*



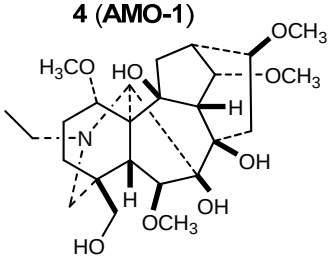
4 (**AMO-1**)



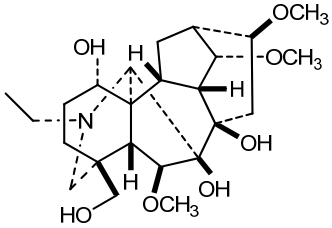
5 (**AMO-2**)



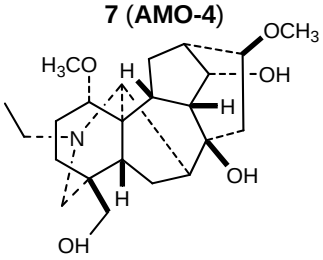
6 (**AMO-3**)



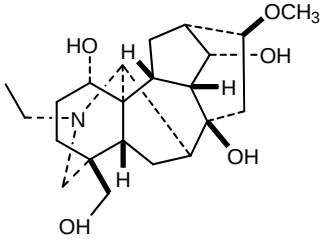
7 (**AMO-4**)



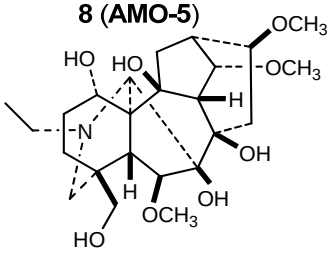
8 (**AMO-5**)



9 (**AMO-6**)

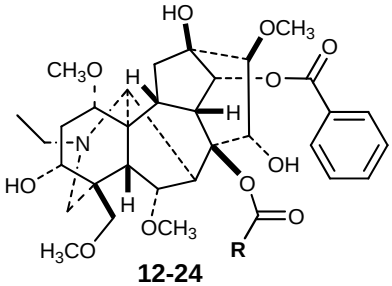


10 (**AMO-7**)



11 (**AMO-8**)

Semisythetic lipo-alkaloids



12-24

R: esterifying fatty acid

Comp. No.	Esterifying fatty acid	Corresponding lipo-alkaloid (BzA = benzoylaconine)
12	lauric	14-BzA-8-*O*-laurate
13	myristic	14-BzA-8-*O*-myristate
14	stearic	14-BzA-8-*O*-stearate
15	palmitoleic	14-BzA-8-*O*-palmitoleate
16	oleic	14-BzA-8-*O*-oleate
17	α-linolenic	14-BzA-8-*O*-α-linolenate
18	γ-linolenic	14-BzA-8-*O*-γ-linolenate
19	eicosanoic	14-BzA-8-*O*-eicosanoate
20	11*Z*-eicosenoic	14-BzA-8-*O*-eicosa-11*Z*-enoate
21	11*Z*,14*Z*- eicosadienoic	14-BzA-8-*O*-eicosa-11*Z*,14*Z*-dienoate
22	8*Z*,11*Z*,14*Z*-eicosatrienoic	14-BzA-8-*O*-eicosa-8*Z*,11*Z*,14*Z*-trienoate
23	eicosapentaenoic	14-BzA-8-*O*-eicosapentaenoate
24	docosahexaenoic	14-BzA-8-*O*-docosahexaenoate

LAs: Semisynthesis and antiphlogistic activity testing

Processed aconite roots are widely used in Eastern medicinal systems, especially in TCM as painkillers and antirheumatic agents. As long as aconitine-type DAs found in unprocessed roots are known to exhibit a broad spectrum of pharmacological activities, including antinociceptive and anti-inflammatory effects *in vitro*, it is noteworthy that LAs are characteristic compounds of both processed and unprocessed aconite drugs. Their amount significantly increases in the course of the traditional processing of the drugs. Aconitine-type alkaloids (e.g. aconitine, hypaconitine, mesaconitine) are highly toxic, in contrast to LAs, which possess significantly less toxicity due to the presence of a long chain fatty acid moiety in the molecules at C-8. In the last decade several analytical aspects of these compounds and processed aconite drugs have been reported, but no detailed pharmacological studies were conducted in connection with their therapeutic relevance; and this phenomenon also means that less attention has been paid so far to the potential role of LAs in the pharmacological effects of processed aconite drugs.

Processing (usually boiling) of crude aconite roots decreases the amount of toxic alkaloids and increases the concentration of LAs. Therefore, toxic aconite alkaloids cannot be responsible for activity, but LAs may be. The fact that the long chain fatty acid residues can reduce the high toxicity of DDAs, while still retaining the otherwise desirable antinociceptive and anti-inflammatory activities is of great importance. However, because of the close structural similarities of compounds substituted with lipoid chains, these alkaloids have not been isolated to date in pure form from natural sources.

With the aim to evaluate the antiphlogistic potential of fatty acid substituted NDAs, a series of 13 aconitine derived LAs (**12**–**24**) as model substances were prepared semisynthetically, and subjected to *in vitro* anti-inflammatory assays in order to gain information about this pharmacological effect of LAs and to conclude some structure-activity relationships concerning the nature of the esterifying fatty acids.

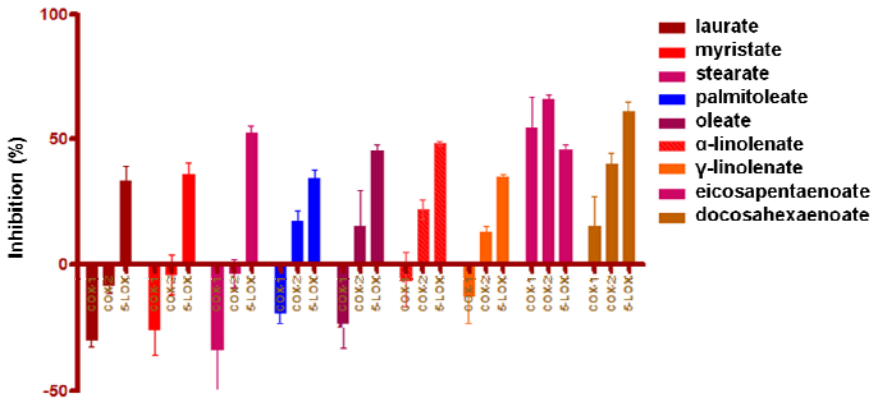


Figure 3. Anti-inflammatory activities of certain LAs

The COX-1, COX-2 and LTB_4 formation inhibitory activities of the compounds were investigated. In the COX-1 assay only compounds substituted with a C_{20} or a C_{22} fatty acid moiety (**19-24**) exhibited moderate activity, while all other LAs (**12-18**) were inactive on the COX-1 enzyme independently to the unsaturation and the length of the substituting fatty acid chain. In the COX-2 inhibition assay a correlation between the grade of unsaturation in the ester group and the enzyme inhibitory activity may be presumed, while regarding the 5-LOX assay a weak correlation could be noted between the activity and the length of the fatty acid chain (for graphical illustration of the activities of certain LAs see **Figure 3**).

Investigation of ion channel inhibitory activities of selected compounds

Because of the fact that in contrast with the exorbitant body of chemical information the common pharmacological knowledge on DAs can still be considered as rather poor, co-operative research programmes to gain further data on the pharmacological characteristics of DAs were initiated. Within the framework of this a selection of DA compounds was subjected to hERG and $Na_v1.2$ channel inhibitory assays. This series included: aconitine (purchased), 14-benzoylaconine-8-*O*-palmitate and pyroaconitine (semisynthetically produced); and acotoxicine, aconosine, dolaconine, delectinine, neolinine, neoline, acotoxinine, songoramine and songorine isolated from *A. toxicum;* acovulparine and septentriodine isolated from *A. vulparia*; delcosine, gigactonine, takaosamine and 14-desacetyl-18-demethylpubescenine from *Consolida orientalis*; and isotalatizidine (**1**, **ANT-1**), 10-hydroxy-8-O-methyltalatizamine (**2**, **ANT-2**), hetisinone (**3**, **ANT-3**) isolated from *A. anthora*, and ajacine (**5**, **AMO-2**), lycoctonine (**6**, **AMO-3**), and swatinine (**7**, **AMO-4**) isolated from *A. moldavicum*.

Although most of the cardiovascular and neurological effects and poisonings with *Aconitum* products are explained by the activation of Na^+ channels, the involvement of the K^+ channels in the pharmacological effects of DAs is not explored systematically. The human ether-à-go-go-related gene (hERG) encodes a cardiac voltage-gated K^+ channel (Kv11.1) that provides the major repolarising current (IKr) in phase 3 of the cardiac action potential. Most drugs that have been shown to increase the QT interval of the ECG at therapeutic doses incorporate IKr inhibition in their spectrum of effects. Prolongation of the QT interval can lead in rare cases to the polymorphic ventricular dysrhythmia called torsade de pointes (TdP) and sudden death. Only one previous study has been reported the hERG channel inhibitory effect of the most commonly available DA, aconitine.

Our data suggest that some structurally and functionally unrelated DAs may block the hERG K^+ channel, and may therefore act in cardiac action potential repolarisation, with prolongation of the QT interval, and increase of the risk of potentially fatal ventricular arrhythmias. The highest hERG blockade activity was observed for the norditerpene diesters aconitine and 14-BzA-8-*O*-palmitate,

which exerted a 44.9 and a 39.6% inhibitory effect, respectively. The further monoesters septentriodine, ajacine (**5**, **AMO-2**), acotoxinine and dolaconine also revealed only moderate K^+ channel potency, with 20.9, 13.0, 6.5 and 8.3% inhibition, respectively. Our results indicate that the substitution with two ester groups is an important structural feature for the K^+ channel activity. However, it can not be stated that the presence of an aryl ester group is required for the exertion of hERG activity, since ajacine (**5**, **AMO-2**) and acotoxinine containing aromatic ester functionalities, do not possess high potency. As concerns the other non-esterified compounds based on the aconitane skeleton, gigactonine and neolinine exhibited marked hERG-inhibiting ability with 38.0 and 35.8% inhibitory activity, respectively. The toxic plasma concentration of aconitine is 30-1000 times lower than its IC_{50} value on hERG channels, and its serious cardiac side effects are generally believed to be a result of Na^+ channel modulation. However, for the other hERG-active alkaloids, the above difference in concentration is potentially small enough to exert their hERG effect during the therapeutic application of aconite drugs, which could result in adverse cardiovascular effects. Our results call the attention to the fact that relatively minor structural differences may cause considerable differences in efficacy; and more importantly clearly indicate that the cardiovascular safety profile of *Aconitum* drugs should be evaluated taking into account the possible effect of DAs on hERG channels.

Unfortunately, sodium channel blockers currently applied with different therapeutic indications discriminate poorly between $Na_v1.x$ subtypes and their tolerable therapeutic indices may rather be the consequence of their use-dependent properties. Another important aspect is that compounds used therapeutically should devoid or have the least minimal activity against the hERG cardiac K^+ channel being involved in cardiac toxicity.

Concerning the relationship between the structural features and the $Na_v1.2$ inhibiting activity of the studied alkaloids it seems relevant that the concomitant presence of a methoxy function at C-1, the presence of an oxygen-containing functionality at C-8, and the presence of a (substituted) aroyl function either at C-14 or at C-18 is necessary, with the parallel requirement that the number of oxygen functionalities in the molecule should be at least 4. Satisfaction of these stipulations can be seen in the case of all significantly (pyroaconitine, ajacine (**5, AMO-2**), delectinine, septentriodine) and moderately active compounds (lycoctonine (**6, AMO-3**), acotoxicine, acovulparine, aconitine). When C-1 methoxy is changed to hydroxyl, the inhibitory effects of the compounds decrease as it can be seen in the case of delectinine (42% inhibition) and takaosamine (3% inhibition). The only, nonetheless very interesting outlier compound is swatinine (10% inhibition), which has a methoxy group at C-1; however, it is substituted with an unusual, extra hydroxyl at C-10. Without this latter substituent, the compounds substitution pattern is identical with acovulparine (with the only structural difference occurring on the *N* atom) (30% inhibition). This surprising, and rather large activity difference allows to hypothesise the importance of hydroxy substitution at C-10 that may

pose some kind of steric hindrance for the molecule when interacting with the channel receptor. However, it cannot be excluded that differences in the substitution of *N* atom may also be of importance regarding this difference in activity. Influence of the position of the aroyl functionality on the inhibitory activity is illustrated by the example of acotoxinine, which was found to be inactive, in spite of the fact that it has an aroyl functionality in the molecule, but it is positioned at C-8, not at C-14 or C-18, as it has been described above. These stipulations seem to be supported by a detailed QSAR analysis of 12 DAs, which revealed that the position of the aroyl/aroyloxy groups at C-4 or C-14 is the major determinant of the analgesic activity. Alkaloids with an aroyl or an aroyloxy group at C-14 exhibited an analgesic potency approximately 30 times higher than that of alkaloids with an aroyloxy group at C-4 in a model of acetic acid-induced writhing in rats. Previously it has also been proven that alkaloids with low affinity for the neurotoxin receptor site 2 of Na^+ channels lack antinociceptive action. As regards the three C_{20} DAs involved in this study only one (hetisinone (**3, ANT-3**)) has been found to exert moderate activity, therefore no relevant deductions may be made concerning the structure-activity relationship of these compounds. It is noteworthy, though to mention that songorine was found practically inactive. Further two aspects to be emphasised are that amongst the four most active compounds only one, delectinine is not esterified (although satisfying all other presumably necessary conditions), and that both diester-type compounds (14-BzA-8-*O*-palmitate and aconitine) have been found to exert only moderate inhibitory activity on the studied particular channel subtype.

Considering our herein results, especially in light of our results on the hERG K^+ channel inhibitory activity of these DAs it can be observed that certain compounds (14-BzA-8-*O*-palmitate, aconitine, gigactonine) exerted noteworthy activity in both pharmacological tests, thus despite their promising $Na_v1.2$ inhibitory activity these compounds should be considered as potentially harmful by causing adverse cardiovascular effects through their hERG effect, nevertheless, some of these compounds show some selectivity over hERG channels. Among the studied active alkaloids particularly those should be further pursued as model substances for pharmacological lead compounds as selective $Na_v1.2$ inhibitors, which exert minor or no hERG activity, i.e. ajacine (**5, AMO-2**) and delectinine.

Analysis of processed TCM aconite drugs for DA content

Traditional processing, which is a generally applied approach in the Far Eastern traditional medicinal systems, provides aconite drugs for human therapeutic use. Practically all analytical works on the topic demonstrated that cautious processing (usually boiling) of crude aconite roots decreases the amount of normal DAs and increases the concentration of LAs resulting in the reduction of toxicity of

the drugs. Quality control criteria of pharmacopoeias are not always sufficient to warrant safety. In case of *Radix aconiti* and *Radix aconiti kusnezoffii*, the DA content is not limited in the Chinese Pharmacopoeia and the warning it stipulates ("be cautious about the unprocessed root taken orally") is not commensurable to the danger of toxicity. In the *Radix aconiti praeparata* and *Radix aconiti kusnezoffii praeparata* monographs, a colorimetric assay is used for the determination of DDAs (required content level should not be >0.15%), and a titrimetric assay for the determination of the total alkaloid content (required level should not be <0.20% of alkaloids, calculated as aconitine). The dosage of these two drugs is 1.5–3 g, which may contain as much as 4.5 mg DDAs. Moreover, for *Radix aconiti lateralis praeparata*, the dose of which is 3–15 g, only the TLC analysis of aconitine is specified. Taking into account that the minimum lethal dose of aconitine is 3–6 mg, it is obvious that only careful processing and quality control may warrant the safety of aconite containing medicinal products. Several homeopathic pharmacopoeias contain a monograph on *A. napellus*, and the essence of methods used for the qualification of its drugs, as demonstrated in the most prominent homeopathic pharmacopoeia, the *German Homeopathic Pharmacopoeia,* is the titrimetric determination of the total alkaloid content. Since the titrimetric determination provides no direct information on the toxic DDA content of aconite roots, more reliable methods are required for the quality control of processed plant material. Therefore the aim of our work was to develop a quick and simple HPLC method for the quality control of aconite roots with comparable or better reliability to those of the previously published methods, to compare the results of titration method with HPLC analysis of the toxic alkaloids.

For analysis, we applied sample preparation and an HPLC method developed by us, along with pharmacopoeial titrimetric measurements. This HPLC method offers a quick and reliable possibility to determine the quantity of the most important toxic alkaloids of *A. carmichaelii* and *A. kusnezoffii* and was developed with the aim of providing a proper analytical tool for pharmacopoeial aconite drug analysis. In most of the commercial samples, toxic alkaloids were undetectable, or only traces were found by the applied HPLC method. However, the fact that in four samples toxic aconite alkaloid levels could be detected above 0.04%, highlights the consideration that these alkaloid contents are high enough to question the safety of the samples concerned. Samples with mesaconitine, aconitine, and hypaconitine content below the HPLC detection limit still contained up to 0.2% alkaloids determined by titration. The comparison of our method with the widely applied titrimetry revealed that alkaloid titration may lead to inaccurate assessment of the toxic DDA content of the samples and confirms that introduction of aconite drugs into European medicine necessitates the application of relevant and validated methods.

5. SUMMARY

Novel scientific results – résumé:

1. Phytochemical investigation of *A. anthora* and *A. moldavicum* resulted in the isolation of 2 new [10-hydroxy-8-*O*-methyltalatizamine (**2**, **ANT-2**) and 1-*O*-demethylswatinine (**11**, **AMO-8**)], and 9 known DAs [hetisinone (**3, ANT-3**), isotalatizidine (**1**, **ANT-1**), delcosine (**4**, **AMO-1**), ajacine (**5**, **AMO-2**), lycoctonine (**6**, **AMO-3**), swatinine (**7**, **AMO-4**), gigactonine (**8**, **AMO-5**), cammaconine (**9**, **AMO-6**) and columbianine (**10**, **AMO-7**)], which – with the exception of one compound – was isolated from these species for the first time; the latter taxon was investigated chemically for the first time;
2. Through extensive 1D and 2D NMR studies structure elucidation and complete and unambiguous ^{1}H and ^{13}C assignments was carried out for all isolated compounds; reassignment, supplementation or revision of missing or incorrect data was performed for the already known compounds;
3. 13 Aconitine-derived LAs were prepared semisynthetically and purified chromatographically by a methodology developed by us;
4. Anti-inflammatory assays with the semisynthetic LAs were carried out on the COX-1, COX-2 and LTB_4 formation inhibition models to establish for the first time certain antiphlogistic structure-activity relationships concerning the effect of the nature of the esterifying fatty acid moiety;
5. Pharmacological bioassays on the hERG and Nav1.2 channels were carried out by using a selected series of DAs to establish for the first time certain structure-activity relationships concerning the effect of the tested DAs on these channels;
6. As a result of these pharmacological assays ajacine (**AMO-2**) and delectinine, which are selective $Na_v1.2$ inhibitors, and at the same time exert minor or no hERG activity were identified as perspective compounds for further pharmacological analyses and as potential lead compounds.
7. Determination of the toxic DA content in 16 authentic processed *Radix aconiti* samples was performed by an HPLC method developed by us, 4 potentially toxic commercial samples were identified; comparison of our results with the widely applied titrimetry revealed the superiority of our method in terms of accuracy and therapeutic relevance.

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The thesis is based on the following publications

1. Forgo P, **Borcsa B**, Csupor D, Fodor L, Berkecz R, Molnár VA, Hohmann J
Diterpene alkaloids from *Aconitum anthora* and assessment of the hERG-inhibiting ability of *Aconitum* alkaloids
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Other publications related to the thesis

5. **Borcsa B**, Fodor L, Csupor D, Forgo P, Molnár VA, Hohmann J
Diterpene alkaloids from *Aconitum moldavicum* and assessment of $Na_v1.2$ sodium channel activity of *Aconitum* alkaloids (*in press, Planta Med* **2014; DOI:** http://dx.doi.org/10.1055/s-0033-1360278)

Presentations held in the theme of the thesis

1. **Borcsa B**, Fodor L, Csupor D, Forgo P, Hohmann J
Assessment of the $Na_v1.2$ sodium channel activity of *Aconitum* diterpene and norditerpene alkaloids
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3. **Borcsa B**, Csupor D, Forgó P, Widowitz U, Bauer R, Hohmann J
Lipoalkaloidok akonitinből történő félszintetikus előállítása és analitikai vizsgálata (Semisynthesis and analytical studies of lipo-alkaloids prepared from aconitine)
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4. **Borcsa B**, Widowitz U, Csupor D, Péter F, Forgo P, Bauer R, Hohmann J
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5. Csupor D, **Borcsa B**, Heydel B, Hohmann J, Zupkó I, Ma Y, Widowitz U, Bauer R
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6. **Borcsa B**, Csupor D, Forgó P, Widowitz U, Bauer R, Hohmann J

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