1st International Electronic Conference on Medicinal Chemistry

2-27 November 2015

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

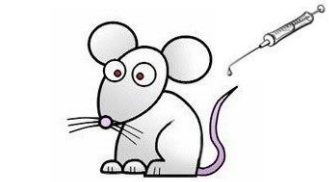
Analgesic and anti-inflammatory activity of diterpenoid alkaloids isolated from the Central Asian species of *Aconitum* and *Delphinium* plants

Firuza Tursunkhodjaeva*, Farkhad Dzhakhangirov

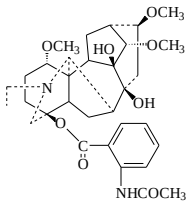
Institute of the Chemistry of Plant Substances, Uzbek Academy of Sciences, 77, Mirzo Ulugbek street, Tashkent, 100170, Uzbekistan

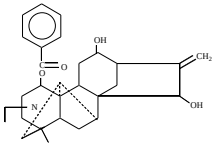
* Corresponding author: ftm40438@gmail.com

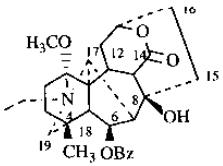
Analgesic and anti-inflammatory activity of diterpenoid alkaloids isolated from the Central Asian species of *Aconitum* and *Delphinium* plants

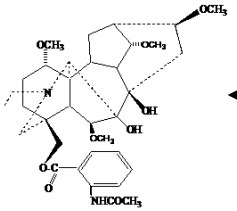


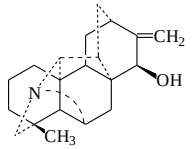
PAIN

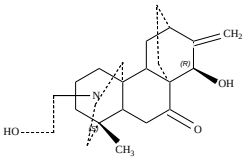


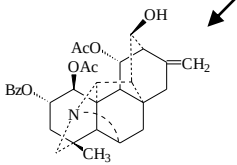




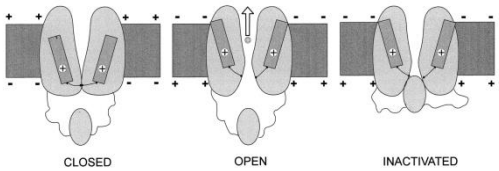






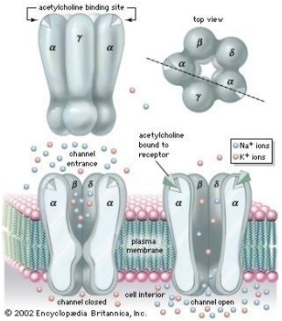


Na+-channels

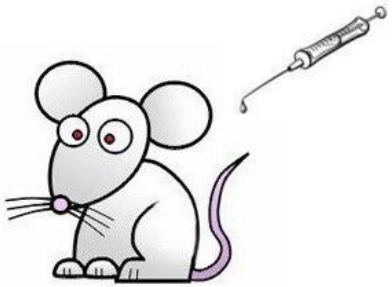


Blockage Activation

N-acetylcholinoreceptors



Blockage



PAIN

Edema

Abstract: In different countries of Europe, Asia and America the plants containing diterpenoid alkaloids have been used on the folk medicine from ancient time. *Aconitum* and *Delphinium* plants (Ranunculaceae family) and their extracts are used in the Eastern medicine at present as antirheumatic, analgesic, anti-inflammatory and other remedies. More 50 species of *Aconitum* (300 worlwide) and 100 species of *Delphinium* (450 worldwide) grow on the territory of Former Soviet Union countries, including Russia, Central Asia and Kazakhstan.

We investigated antinociceptive and anti-inflammatory activity of individual diterpenoid alkaloids isolated from *Aconitum* and *Delphinium* species widespread in the Central Asia and revealed 25 promising substances. Antinociceptive activity was investigated in the conventional tests for displaying analgesics with central mechanisms of analgesia (hot plate) and peripheral mechanisms (acetic writhing, local anesthesia). Anti-inflammatory activity was studied in rat formalin test.

By comparison of antinociceptive activity of investigated alkaloids and underly mechanisms of their pharmacological action we divided them on the following types: activators of potential-gated Na^+-channels of neurons cause shifting of the threshold of Na^+-current towards membrane hyperpolarization and destroy neuronal conductivity; blockers of potential-gated Na^+-channels cause inhibition of the fast intake Na^+-current without changing of its activation threshold; blockers of N-cholinoreceptors.

Keywords: *Aconitum*, *Delphinium*, diterpenoid alkaloids, antinociceptive, anti-inflammatory activity

In different countries of Europe, Asia and America the plants containing diterpenoid alkaloids have been used on the folk medicine from ancient time.

Aconitum and *Delphinium* plants (Ranunculaceae family) and their extracts are used in the Eastern medicine at present as antirheumatic, analgesic, anti-inflammatory and other remedies.

More **50** species of *Aconitum* (**300** worlwide) and **100** species of *Delphinium* (**450** worldwide) grow on the territory of Former Soviet Union countries, including Russia, Central Asia and Kazakhstan. Totally **700** diterpenoid alkaloids have been isolated from plants over the world.

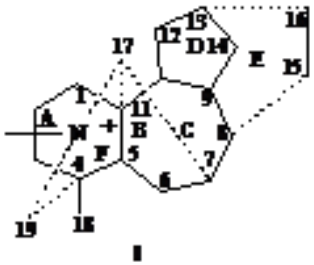
Chemical composition of **32** species of *Aconitum* and **20** species of *Delphinium* growing in the Former Soviet Union countries has been studied in the Laboratory of Alkaloid Chemistry (Institute of the Chemistry of Plant Substances, ICPS, Uzbekistan).

131 diterpenoid alkaloids were isolated from *Aconitum* plants (**72** of them new),
66 diterpenoid alkaloids – from *Delphinium* species (**33** of them new).

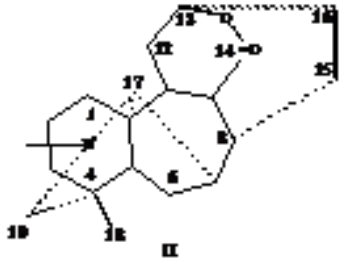
Pharmacological activity and toxicology of these diterpenoid alkaloids (including analgesic and anti-inflammatory activity) has been investigated in the Department of Pharmacology and Toxicology, ICPS.

Diterpenoid alkaloids classification

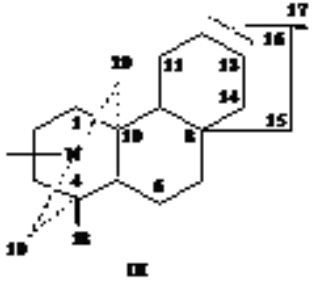
by skeleton



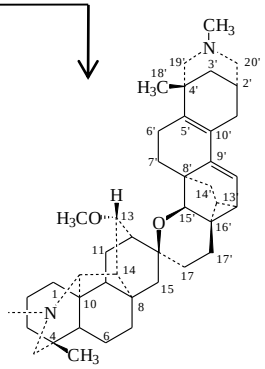
Lycoctonine



Heteratisine



Perhydrophenantrene



Bimolecular

Contain C18 or C19 atoms in the skeleton

Contains C20 atoms in the skeleton

by nature of substituents:

Aminoalcohols

Esters of aminoalcohols

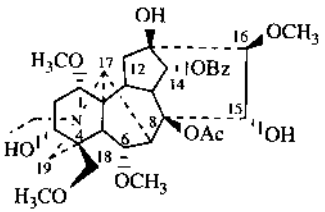
- Esters of aliphatic acids
- Esters of aromatic acids
- Esters of aliphatic and aromatic acids

Investigated substances

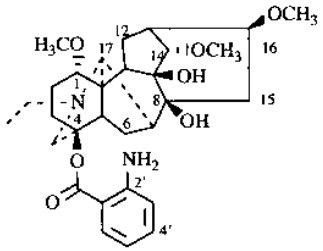
Alkaloid	Source	Alkaloid	Source
Lycoctonine skeleton			
Aconitine (1)	A.altaicum, A.baicalense, A.chasmanthum, A.ferox, A.karacolicum, A.nasutum, A.nemorum, A.songoricum, A.tauricum, A.tuberosum, A.turczaninowii, A.volubile	**Ranaconitine (8)**	A.orientale, A.rubicundum, A.septentrionale
Mesaconitine (2)	A.altaicum, A.firmum, A.sczukinii, A.tauricum, A.tokii, A.tuberosum	**Ajacine (9)**	A.rubicundum, A.zeravschanicum, D.orientale
Aconifine (3)	A.coreanum, A.karacolicum	**1-O-Benzoylisotalatisidine (10)**	Semisynthetic
Lappaconitine (4)	A.leucostomum, A.orienlale, A.panuculatum, A.septentrionale , A.talassicum	**14-O-Benzoyltalatisamine (11)**	A.nemorum
N-Deacetyllappaconitine (5)	A.leucostomum, A.orienlale, A.septentrionale	**6-O-Benzoyleldelidine (12)**	Semisynthetic
Sepaconitine (6)	A.leucostomum, A.septentrionale	**Browniine (13)**	D.biternatum, D.corumbosum, D.ilience, D.rotundifolium
N-Acetylsepaconitine (7)	A.leucostomum		

Investigated substances

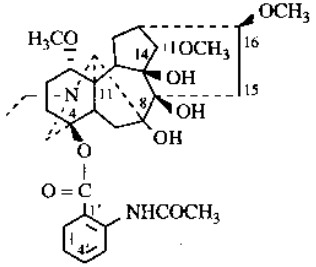
Alkaloid	Source	Alkaloid	Source
Heteratisine skeleton			
6-O-Benzoylheteratisine (14)	A.zeravschanicum, A.heterophyllum	**6-O-Furoylheteratisine (15)**	Semisynthetic
Perhydrophenantrene skeleton			
Talatisine (16)	A.talassicum	**Acorine (21)**	A.coreanum
Dihydroatisine (17)	A.zeravschanicum	**Zeravschanisine (22)**	A.zeravschanicum
Benzoylatisine azomethine (18)	A.zeravschanicum	**Nominine (23)**	A.zeravschanicum
Atidine (19)	A.zeravschanicum A.heterophyllum	**Hetisine (24)**	A.zeravschanicum
Tadzhaconine (20)	A.zeravshanicum, A.firmum, A.anthoroideum	**1-O-Benzoylnapelline (25)**	Semisynthetic



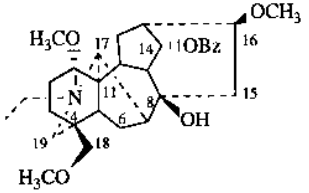
1



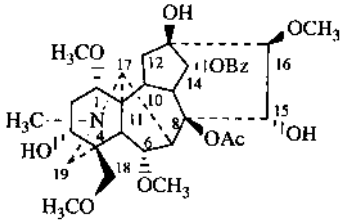
5



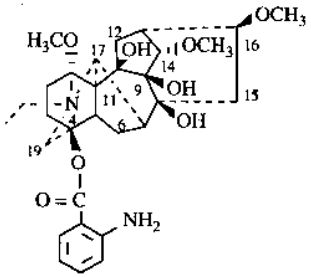
8



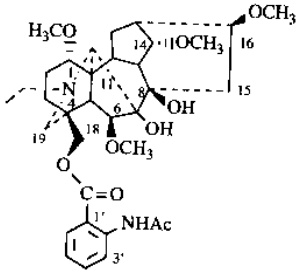
11



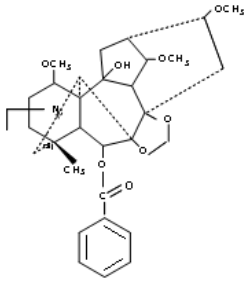
2



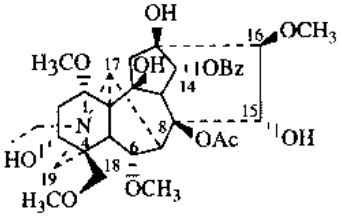
6



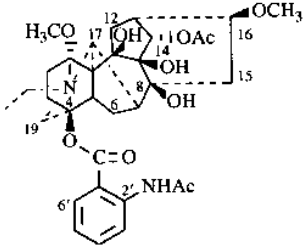
9



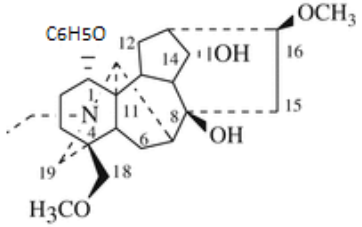
12



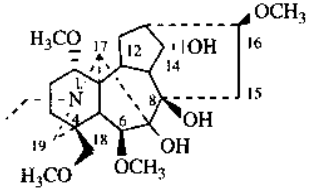
3



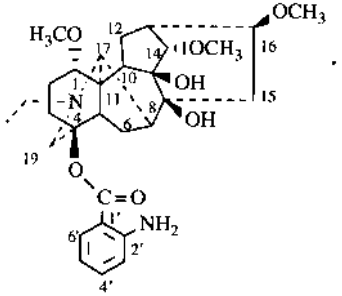
7



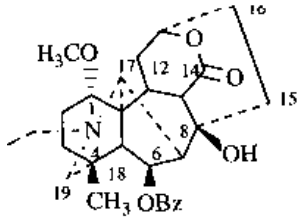
10



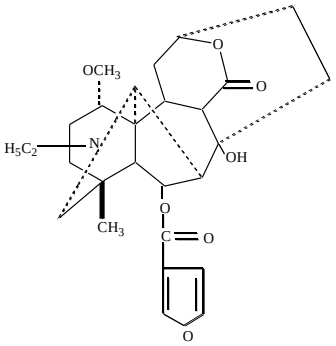
13



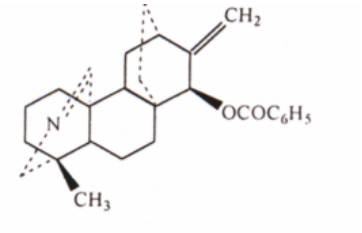
4



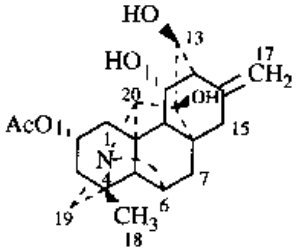
14



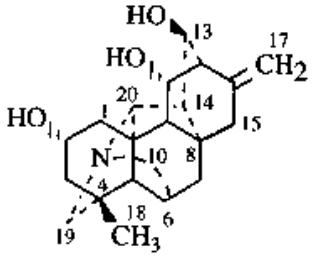
15



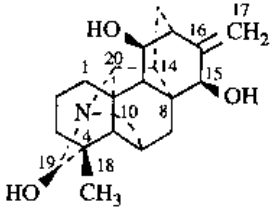
18



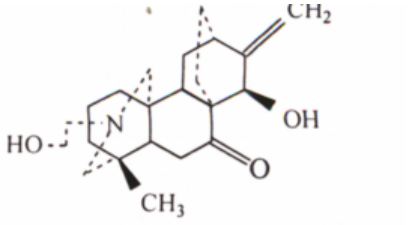
21



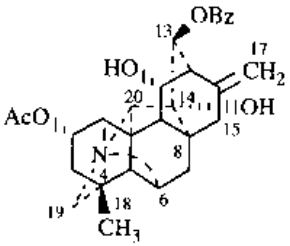
24



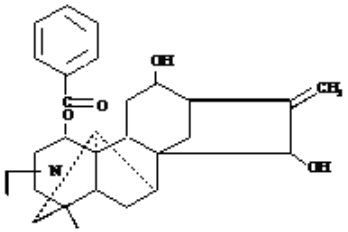
16



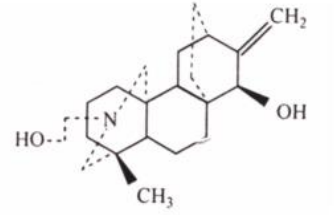
19



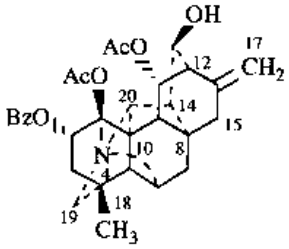
22



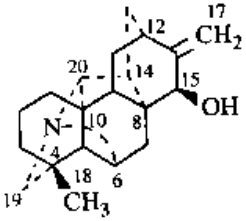
25



17



20



23

Methods

Analgesic activity

- Hot plate test (displaying analgesics with central mechanisms of analgesia)
- Acetic writhing test (displaying analgesics with peripheral mechanisms of analgesia)

Anti-inflammatory activity

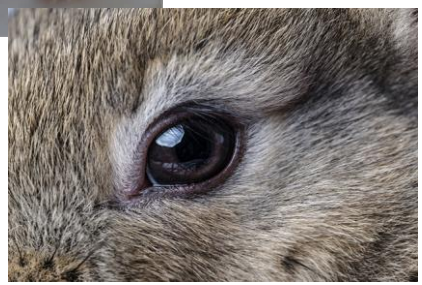
- Rat formalin test

Electrophysiology

- **Patch clamp and intercellular perfusion techniques**

Local anesthetic activity

- **Rabbit eye corneal anesthesia**



Results and discussion

Analgesic activity of the most promising diterpenoid alkaloids in acetic writhing test (mice)

Substance	ED_{50}, mg/kg s.c.	LD_{50}, mg/kg i.p.	Substance	ED_{50}, mg/kg s.c.	LD_{50}, mg/kg i.p.
Morphine	1.3	531.0 (s.c.)	**6-O-Benzoyleldelidine**	4.8	66.0
Aconitine	0.1	0.45	**Ajacine**	4.8	35.4
Aconifine	0.15	1.25	**Atidine**	5.0	>150
6-O-Benzoylheteratisine	2.4	21.5	**Sepaconitine**	5.6	62.2
N-deacetyllappaconitine	2.8	35.0	**6-O-Furoylheteratisine**	5.9	68.0
Lappaconitine	3.1	15.5	**1-O-Benzoylisotalatisidine**	7.3	120.0
Ranaconitine	3.4	11.6	**1-O-Benzoylnapelline**	8.3	135.0
Tadzhaconine	3.4	>100	**14-O-Benzoyltalatisamine**	11.2	122.5
Zeravschanisine	3.5	160.0	**Nominine**	11.0	>150
Dihydroatisine	4.0	88.0	**Sodium metamizole**	53.0	2470.0
Hetisine	4.5	27.0	**Acetylsalicylic acid**	205.0	1020.0 (s.c.)

Analgesic activity of the most promising diterpenoid alkaloids in hot plate test (mice)

Substance	Dose, mg/kg s.c.	Increasing of pain sensation threshold, X times in comparison to the control in 30 min. after alkaloids injection	Substance	Dose, mg/kg s.c.	Increasing of pain sensation threshold, X times in comparison to the control in 30 min. after alkaloids injection
Mesaconitine	0.08	1.50	Acorine	5	1.50
Aconitine	0.15	1.56	Benzoylatisine azomethine	5	1.50
Aconifine	0.15	1.50	Zeravschanisine	5	1.44
Tadzhaconine	5	2.32	Atidine	5	1.40
Ajacine	5	2.30	1-O-Benzoyltalatisidine	5	1.34
Lappaconitine	5	2.30	Nominine	5	1.30
Ranaconitine	5	2.23	Morphine	1	1.00
N-acetylsepaconitine	5	2.00		3	1.52
Dihydroatisine	5	2.00		5	2.70
N- Deacetyllappaconitine	5	1.80	Sodium metamizole	100	1.20
6-O-Benzoylheteratisine	5	1.60	Acetylsalicilic acid	100	1.09

Anti-inflammatory activity of promising diterpenoid alkaloids in 1/10 LD_{50} dose (rats)

Substance	Dose, mg/kg i.p.	Anti-inflammatory effect, %	Substance	Dose, mg/kg i.p.	Anti-inflammatory effect, %
Sodium metamizole	25.0	60.0	Aconitine	0.04	12.8
1-Benzoylisotalatisidine	12.0	57.87	Benzoylatisine azomethine	4.0	12.6
1-O-Benzoylnapelline	14.0	39.5	Lappaconitine	1.6	12.3
Acorine	5.4	39.0	Browniine	45.0	11.3
N-deacetyllappaconitine	3.0	31.0	Ranaconitine	1.2	4.8
Benzoylheteratisine	2.0	30.6	Nominine	5.0	0.0
Ajacine	3.6	28.8	Zeravschanisine	5.0	0.0
Dihydroatisine	9.0	24.6	Atidine	5.0	0.0
Hetisine	2.7	15.0	Acetylsalicilic acid	50.0	51.0

Local anesthetic effect of investigated substances on rabbit eye cornea

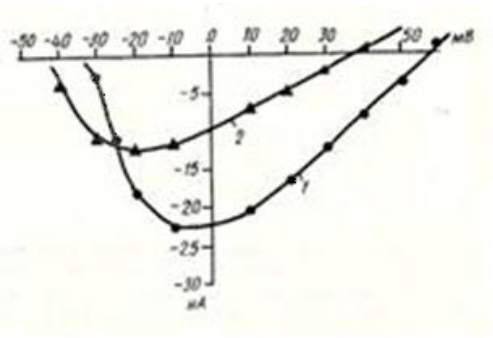
Substance	Concentration, %	Anesthesia duration, min	Onset time, min	Substance	Concentration, %	Anesthesia duration, min	Onset time, min
6-O-Benzoyleldelidine	0.05	45±1.85	5.0±0.39	**Zeravschanisine**	0.25	86.0±2.2	2.4± 0.23
	0.50	210±3.28	1.0±0		0.50	94.0±2.5	2.2±0.18
Tadzhaconine	0.05	40.0±0.93	3.7±0.21	**Benzoylatisine azomethine**	0.25	62.0±1.01	4.0±0.25
	0.50	210+3.97	1.0±0.1				
Ranaconitine	0.10	158±3.6	7.0±0.52	**Dihydroatisine**	0.25	30.2±1.2	1.0±0.1
	0.25	284±4.3	4.8±0.18		0.50	34.0±1.76	3.0±0.55
Lappaconitine	0.10	150±3.5	8.0±0.62	**1-Benzoylisotalatisidine**	0.25	10±0.53	5±0.35
	0.50	310±4.32	5.0±0.16		0.50	14±0.88	5±0.28
Sepaconitine	0.10	58±1.98	4.1±0.28	**Atidine**	0.50	26±1.3	8.4±0.21
	0.50	75±2.12	3.6±015		1.00	55±1.9	2.3±0.17
N-deacetyllappaconitine	0.10	50±0.66	5.5±0.28	**Nominine**	0.50	14±1.6	8.1±0.24
	0.50	100±3.17	3.1±0.26		1.00	34±1.24	2.2±0.18
6-O-Furoylheteratisine	0.10	48±0.93	2.7±0.07	**Hetisine**	0.50	12±1.6	7.4±0.36
	0.50	180±2.91	1.0±0		1.00	25±1.41	2.5±0.25
1-O-Benzoylnapelline	0.10	36.2±0.92	2.3±0.13	**14-O-Benzoyltalatisamine**	0.5	0	0
	0.50	75.2±1.8	1.0±0.08		1.00	10±0.53	3.9±0.26
N-acetylsepaconitine	0.25	196±2.4	8.1±0.28	**Cocaine**	0.10	0	0
	0.50	340±4.5	4.3±0.13		0.25	11.5±0.28	1.16±0.03
					0.50	13.6±0.44	1.0±0
6-O-Benzoylheteratisine	0.25	190±3.05	2.7±0.06	**Dicaine**	0.25	43±1.3	1.0±0
	0.50	210±3.97	1.0±0		0.50	52±0.51	1.0±0

Electrophysiological mechanisms of investigated diterpene alkaloids

Alkaloid	Mechanism	Alkaloid	Mechanism
Aconitine	Na^+-channel activator	**Benzoylatisine azomethine**	Na^+-channel blocker
Mesaconitine	Na^+-channel activator	**Tadzhaconine**	Na^+-channel blocker
Aconifine	Na^+-channel activator	**Acorine**	Na^+-channel blocker
Lappaconitine	Na^+-channel blocker	**Zeravschanisine**	Na^+-channel blocker
N-Deacetyllappaconitine	Na^+-channel blocker	**6-O-Benzoylheteratisine**	Na^+-channel blocker
Sepaconitine	Na^+-channel blocker	**6-O-Furoylheteratisine**	Na^+-channel blocker
N-Acetylsepaconitine	Na^+-channel blocker	**Hetisine**	Na^+-channel blocker
Ranaconitine	Na^+-channel blocker	**1-O-Benzoylnapelline**	Na^+-channel blocker
1-O-Benzoylisotalatisidine	Na^+-channel blocker	**Browniine**	nAChR blocker
14-O-Benzoyltalatisamine	Na^+-channel blocker	**Atidine**	nAChR blocker
6-O-Benzoyleldelidine	Na^+-channel blocker	**Nominine**	nAChR blocker
Talatisine	Na^+-channel blocker	**Ajacine**	nAChR blocker
Dihydroatisine	Na^+-channel blocker		

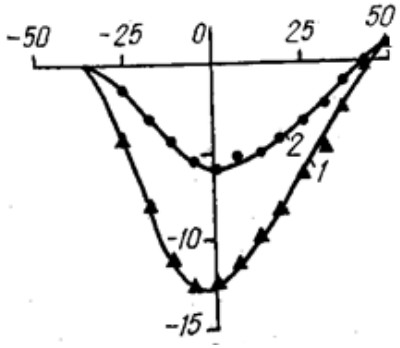
Effects of investigated diterpenoid alkaloids on intake sodium current in isolated neurons

Na+-channel activators



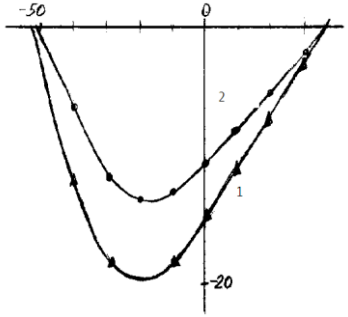
Effects of aconitine (10 μM/l) on intake sodium current of isolated neurons of rat trigeminal ganglia
1 – control solution
2- aconitine-containing solution

Na+-channel blockers



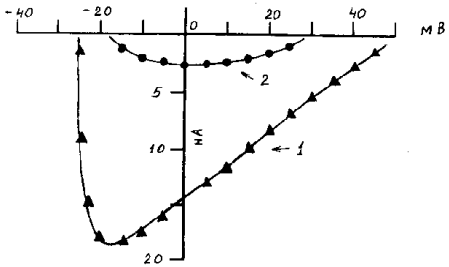
Effects of lappaconitine (10 μM/l) on intake sodium current of isolated neurons of rat trigeminal ganglia
1 – control solution
2- lappaconitine-containing solution

N-acetylcholinoreceptor blockers



Effects of ajacine (10 μM/l) on intake sodium current of isolated neurons of rat spinal ganglia
1 – control solution
2- ajacine-containing solution

Local anesthetics



Effects of dicaine (100 μM/l) on intake sodium current of isolated neurons of rat trigeminal ganglia
1 – control solution
2- dicaine-containing solution

Main characteristic feature attributed to the electrophysiological action of Na+-channel blocking diterpenoid alkaloids is the absence both of sodium current activation threshold shifts and dependence of blocking effect from voltage and stimulus rates.

It may be concluded that the antinociceptive (analgesic and local anesthetic) activity of the investigated diterpenoid alkaloids is based on braking of impulse conductivity on different stages of passing through nociceptive pathways. Analyzing of electrophysiological mechanisms of promising substances shown that the sodium channels of central and peripheral neurons are their main target sites. The investigated substances may be divided on following types:

- Activators of voltage-gated Na^+-channels altering selectivity of ion pore due to binding to site 2 of Na^+-channel. They induce a neuronal block by permanent cell hyperpolarization;

- Blockers of neuronal voltage-gated Na^+-channels by interacting to BTX-sensitive site. They inhibit the fast inward Na^+-current by ion pore bridging;

- Blockers of ligand-gated Na^+-channels (N-acetylcholinoblockers) that destroy cholinergic transmission and downstream Na^+-current. They selectively interact to nAChRs.

Classification, properties and pharmacological targets of voltage-gated sodium channels

From: WILLIAM A. CATTERALL, ALAN L. GOLDIN, AND STEPHEN G. WAXMAN. International Union of Pharmacology. XLVII. Nomenclature and Structure-Function Relationships of Voltage-Gated Sodium Channels. *Pharmacol Rev* 57:397–409, 2005

Channel name	Ion selectivity	Location	Pharmacological significance
Na_v 1.1	$Na^+ > K^+ > Ca^{2+}$	Central neurons; cardiac myocytes	Site of action of antiepileptic drugs
Na_v 1.2	$Na^+ > K^+ > Ca^{2+}$	Central neurons	Site of action of antiepileptic drugs
Na_v 1.3	$Na^+ > K^+ > Ca^{2+}$	Central neurons; cardiac myocytes	Site of action of antiepileptic drugs
Na_v 1.4	$Na^+ > K^+ > Rb^+ > Cs^+$	Skeletal muscles	Target of local anesthetics used to treat myotonia
Na_v 1.5	$Na^+ > K^+ > Ca^{2+}$	Cardiac myocytes, skeletal muscle, certain brain neurons	**Site of action of antiarrhythmic drugs**
Na_v 1.6	Na^+	Somatodendritic distribution in output neurons of the cerebellum, cerebral cortex, and hippocampus; Purkinje cells in the cerebellar granule cell layer; brainstem and spinal cord, astrocytes, and Schwann cells; DRG; nodes of Ranvier of sensory and motor axons in the PNS; nodes of Ranvier in the CNS	Potential target for antiepileptic and **analgesic drugs**
Na_v 1.7	Na^+	All types of DRG neurons, sympathetic neurons, Schwann cells, and neuroendocrine cells	**Probable target of local anesthetics in the peripheral nervous system**
Na_v 1.8	Na^+	Small and medium-sized DRG neurons and their axons	**Potential target for analgesic drugs**
Na_v 1.9	Na^+	c-type DRG neurons, trigeminal neurons and their axons; preferentially expressed in nociceptive DRG neurons	**Potential target for analgesic drugs**

Comparison of analgesic, anti-inflammatory, local anesthetic, N-cholinoblocking* and antiarrhythmic ** activity of studied diterpenoid alkaloids doesn't display a direct correlation. Presence of one type of studied pharmacological effects not indicated presence of other ones. It may be supposed that the realization of the abovementioned types of pharmacological activity involve modulation of different types of sodium channels, and the investigated diterpenoid alkaloids have different affinity to these channels.

Thus, it was revealed that TTX-sensitive $Na_v1.5$ being localized in cardial myocytes and skeletal muscles is a target of antiarrhythmic drugs action. The main site of local anesthetics action is $Na_v1.7$ distributed in central and peripheral neurons. Realization of the said pharmacological effects depends on inhibition of the fast intake sodium current.

Analgesic activity realization is attributed to interaction of a ligand to slow inactivated TTX-resistant sodium channels $Na_v1.8$ and $Na_v1.9$, as well as TTX-sensitive $Na_v1.6$. It is supposed that $Na_v1.8$ and $Na_v1.9$ in DRG neurons may be modulated by mediators of inflammation (prostaglandins, serotonin)*** in hyperalgesia caused by inflammation.

The item of studied diterpenoid alkaloids interaction to different sodium channel types needs a deep investigation in future.

* F. N. Dzhakhangirov, I. A. Bessonova**. Alkaloids of *Aconitum coreanum*. X. Curare-Like Activity-Structure Relationship**. Chemistry of Natural Compounds, January 2002, Volume 38, Issue 1, pp 74-77

F. N. Dzhakhangirov, M. N. Sultankhodzhaev, B. Tashkhodzhaev, B. T. Salimov. **Diterpenoid alkaloids as a new class of antiarrhythmic agents. Structure-activity relationship. Chemistry of Natural Compounds, March 1997, Volume 33, Issue 2, pp 190-202

*** MICHAEL S. GOLD. **Tetrodotoxin-resistant Na+ currents and inflammatory hyperalgesia.** Proc. Natl. Acad. Sci. USA, Vol. 96, pp. 7645–7649, July 1999

Conclusions

1. Antinociceptive (analgesic, local anesthetic) and anti-inflammatory action of diterpenoid alkaloids isolated from the Central Asian species of *Aconitum* and *Delphinium* plants have been investigated. It was revealed 25 promising substances with high analgesic and anti-inflammatory activity.

2. It was shown that analgesic activity of investigated substances increases in aminoalcohols – O-aromatic substituted aminoalcohols range.

3. As analyzing of electrophysiological mechanisms of investigated diterpenoid alkaloids shown, the sodium channels of central and peripheral neurons are the main targets of their action. We divided investigated substances on the following types: activators of potential-gated Na^+-channels of neurons cause shifts of the Na^+-current threshold towards membrane hyperpolarization and destroy neuronal conductivity; blockers of potential-gated Na^+-channels cause inhibition of the fast intake Na^+-current; blockers of N-acetylcholinoreceptors of CNS.

Acknowledgments

Authors express gratitude to Prof. B.T.Salimov and Dr. M.N.Sultankhodjaev (Laboratory of Alkaloids Chemistry, ICPS AS RUz) for providing the investigated chemical substances.