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SEARCH FOR AND STUDY OF CURAREMIMETIC DRUGS AMONG THE DITERPENE ALKALOIDS

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A number of diterpene alkaloids (DAs) and their derivatives have been investigated for curaremimetic activity. Their structure–activity relationships and the mechanisms of their action have been studied. Structural fragments of the DAs that are responsible for curaremimetic properties have been revealed. Among the compounds studied, nudicauline and lycaconitine are superior to the curaremimetic drugs melliktin and condelphine.

Drugs interacting selectively with the H-cholinoreceptors of the central nervous system, the vegetative ganglia, and the neuromuscular synapses are widely used in experimental and practical medicine. The demand for drugs of this type with different action spectra and mechanisms is extremely great, since with their use it is possible to control many functions of the internal organs and to change the tonus of skeletal muscles and also to act on the processes involved in the interneuron transmission of excitation in the central nervous system.

Diterpene alkaloids are of interest as objects in the search for new curaremimetic drugs with an enteral action, since, being tertiary amines, they are absorbed in the gastrointestinal tract, and, acting on the peripheral H-cholinoreceptors and freely penetrating through the blood–brain barrier, they exert an action on the central nervous system.

Investigations performed as early as the 1950s-1960s have shown that such alkaloids from plants of the genus *Delphinium* as lycoctonine (delsine), delsemine, avadharidine, methyllycaconitine, condelphine, and elatine possess a pronounced curaremimetic action which is revealed on injection into the stomach [1-3].

At the present time, methyllycaconitine hydriodide (melliktin/"mellictine") is being used in medicine as a curaremimetic in the treatment of various neurological and other diseases connected with an increased tonus of the skeletal musculature [4]. The structural elements responsible for the curare-like activity of the lycoctonine alkaloids have been discussed in a review [3].

In recent years, as the result of vigorous progress in the field of diterpene alkaloid chemistry, a large number of new compounds have been isolated and their structures have been determined. In view of this, we considered it desirable to carry out a search for new muscle relaxants, to study their structure–activity relationships and action mechanisms, and to determine the prospects of their use in practical medicine and biology.

SCREENING AND THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND CURAREMIMETIC ACTION AMONG DITERPENE ALKALOIDS

We have investigated a series of diterpenoid compounds isolated from plants of the genera *Aconitum* and *Delphinium* and based mainly on lycococtonine, heteratisine, atisine, hetisine, denudatine and veatchine skeletons, and their modified analogs.

The results of the investigations showed that the capacity for exhibiting a muscle-relaxing action is possessed by alkaloids with various types of skeletons. The most active compounds were found among alkaloids with the lycoctonine skeleton, while atisine and heteratisine compounds were less active.

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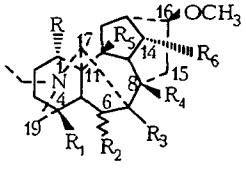
The amino alcohols of the lycoctonine alkaloids, the molecules of which contain a tertiary nitrogen atom and hydroxy and methoxy groups in various positions, exhibited a weaker curaremimetic activity than their etherified and esterified analogs, The most active among them — neoline (**1**) [5], isotalatisine (**2**) [6], karakoline (**3**) [7], and delsoline (**4**) [8] — caused the inclined head syndrome (IHS) in rabbits and neuromuscular transmission block (NMTB) from the sciatic nerve to the gastrocnemius muscle in anesthetized cats in doses of 20-30 mg/kg (intravenously). The disturbance of neuromuscular transmission was of the antidepolarizing type [9, 10].

The amino alcohols exhibited a considerably more pronounced hypotensive and ganglion-blocking action than curaremimetic action. They caused suppression of conduction in sympathetic ganglia in doses considerably smaller than those causing neuromuscular transmission block.

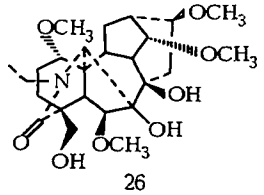
Acetyl derivatives of lycoctonine alkaloids possessed a more pronounced curaremimetic activity, which depended to a considerable degree on the mutual positions of the acetyl and hydroxy groups. Thus 14-acetyltalatisamine (**5**) [7], 14-acetylbrownine (**6**) [12], 1-acetylkarakoline (**7**), 8,14-diacetyltalatisamine (**8**) [13], and eldeline (**9**) [14] were 5-10 and more times less active than condelphine (**10**) [15]. The most active among them proved to be 14-acetylvirescenine (**11**) [15], with an action not inferior to that of condelphine.

The most pronounced muscle relaxant action was exhibited by lycoctonine alkaloids containing an aromatic fragment as an acyl substituent. At the same time, the nature of the substituent and its position are of great importance. The most active proved to be C-18—O-substituted esters of lycoctonine and of delectine. These compounds caused the IHS in rabbits and NMTB in cats in a range of doses of from 1 to 10 mg/kg. As a rule, when the structure of the aromatic fragment was made more complicated the curaremimetic action was enhanced. The muscle relaxant effect of the most active compounds — lycaconitine (**12**) and nudicauline (**13**) [17] — was superior to that of condelphine and melliktin.

Alkaloids containing an aromatic acyl residue in a different position (C-1, C-4, C-6, or C-14), showed a fall in curaremimetic properties and the appearance of a qualitatively different direction of their pharmacological action — cardiotonic. As a result, on the intravenous injection of increasing doses of these substances into cats until changes were reached in neuromuscular transmission, cardiac arrhythmia and hypotension developed and death of the animals ensued.



1. $R=R_4=R_6=OH$, $R_1=CH_2OCH_3$, $R_2=\alpha OCH_3$ (Here and below, unidentified R represents H)
2. $R=R_4=R_6=OH$, $R_1=CH_2OCH_3$
3. $R=R_4=R_6=OH$, $R_1=CH_3$
4. $R=R_3=R_4=OH$, $R_1=CH_2OCH_3$, $R_2=\beta OCH_3$, $R_6=OCH_3$
5. $R=OCH_3$, $R_1=CH_2OCH_3$, $R_4=OH$, $R_6=OCOCH_3$
6. $R=OCH_3$, $R_1=CH_2OCH_3$, $R_2=\beta OCH_3$, $R_3=R_4=OH$, $R_6=OCOCH_3$
7. $R=OCOCH_3$, $R_1=CH_3$, $R_4=R_6=OH$
8. $R=OCH_3$, $R_1=CH_2OCH_3$, $R_4=R_6=OCOCH_3$
9. $R=R_6=OCH_3$, $R_1=CH_3$, $R_2=\beta OCOCH_3$, $R_3{+}R_4=O\text{-}CH_2\text{-}O$, $R_5=OH$
10. $R=R_4=OH$, $R_1=CH_2OCH_3$, $R_6=OCOCH_3$
11. $R=R_3=R_4=OH$, $R_1=CH_2OCH_3$, $R_6=OCOCH_3$
14. $R=R_3=R_4=OH$, $R_1=CH_2OH$, $R_2=\beta OCH_3$, $R_6=OCH_3$
15. $R=R_5=OCH_3$, $R_1=CH_2OH$, $R_2=\beta OCH_3$, $R_3=R_4=OH$
16. $R=OCH_3$, $R_1=CH_2OH$, $R_2=\beta OCH_3$, $R_3=R_4=R_6=OH$
25. $R=OCH_3$, $R_1=CH_2OCH_3$, $R_4=R_6=OH$



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It follows from the facts given that the nature and position of the aromatic nucleus exert a considerable influence on the pharmacodynamics of curaremimetic substances. They can change the mechanism of their action, their activity, and the main direction of their pharmacological effect.

TABLE 1. Toxicities and H-Cholinolytic Activities of Some Diterpene Alkaloids

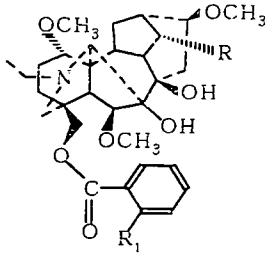
Compound	LD_{50}, mg/kg		Doses (mg/kg) causing on i/v injection into cats		EC_{50}, g/ml (suppression of the reaction of the frog *m. rectus abdominis* to acetylcholine)
	в/в	в/б	NMCB by 50-100%	relaxation of the third eyelid, ED_{50}	
Amino alcohols					
Neoline (**1**) [5]	69.0	244.5	17–20	1.5	$7 \cdot 10^{-6}$
Isotalatisidine (**2**) [6]	40.1	170.0	25–30	3.0	$8 \cdot 10^{-6}$
Karakoline (**3**) [7]	51.5	300.0	30–35	3.2	$9 \cdot 10^{-6}$
Delsoline (**4**) [8]	175.0	550	25–30	4.2	$5 \cdot 10^{-6}$
Gigactonine (**14**) [18]	88.0	–	18–25	2.4	$5 \cdot 10^{-6}$
Lycoctonine* (**15**) [14]	170.0	>600	>70	12	$7 \cdot 10^{-5}$
Delectinine* (**16** [14]	130.0		>70	18.4	$4 \cdot 10^{-4}$
Alkaloids with acetyl substituents					
Condelphine (**10**) [15]	18.5	–	7–10	1.5	$8.2 \cdot 10^{-7}$
14-Acetylvirescenine (**11**) [15]	18.0	–	6–9	1.6	$7 \cdot 10^{-7}$
Alkaloids with aromatic ester substituents					
Melliktin (**17**) [14]	3.9	19.5	2–2.5	0.3	$2 \cdot 10^{-5}$
Lycaconitine (**12**) [16]	2.6	12.5	1.25–2.7	0.25	$1 \cdot 10^{-5}$
Nudicauline (**13**) [17]	1.8	13.0	1–1.5	0.22	$7 \cdot 10^{-6}$
Anthranoyllycoctonine (**18**) [19]	20.1	95.0	5–7	2	$7.5 \cdot 10^{-6}$
Puberaconitine (**19**) [20]	22.5	–	7–10		
Delectine (**20**) [21]	35.8	>100	10–15	8	$3.5 \cdot 10^{-5}$
O-Acetyldelectine (**21**) [22]	15.5	>50	10	3.5	$7.5 \cdot 10^{-6}$
N-Acetyldelectine (**22**) [23]	25.3	>100	10–15	7	$6 \cdot 10^{-5}$
N,O-Diacetyldelectine (**23**) [21]	12.5	>50	5–7	1.3	$8.1 \cdot 10^{-6}$
Ajacine (**24**) [20]	9.0	35.4	5–7	1.8	$9 \cdot 10^{-6}$

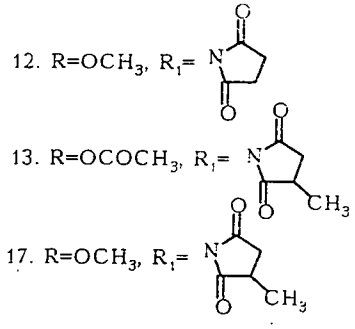
*Curaremimetic activities of compounds (**15**) and (**16**) are given for comparison with the acylated derivatives.

The most active substances revealed as the result of the screening of each of the groups considered are shown in Table 1. The most pronounced curaremimetic activity is possessed by the perchlorates of lycaconitine and nudicauline, the activities of which are superior to those of condelphine and melliktin.

Analysis of the structure–curaremimetic activity relationship in the series of lycoctonine alkaloids has enabled us to derive the following laws:

— curaremimetic activity increases to a considerable degree in compounds with a hydroxy substituent at C-1. Thus, the alkaloids isotalatisidine (**2**) and gigactonine (**14**) are twice as active as talatisamine (**25**) and lycoctonine (**15**), respectively; and,





12. R=OCH_3, R_1=

13. R=$OCOCH_3$, R_1=

17. R=OCH_3, R_1=

18. R=OCH_3, R_1=NH_2
19. R=OCH_3, R_1=$NHCO(CH_2)_2COOH$
20. R=OH, R_1=NH_2
21. R=$OCOCH_3$, R_1=NH_2
22. R=OH, R_1=$NHCOCH_3$
23. R=$OCOCH_3$, R_1=$NHCOCH_3$
24. R=OCH_3, R_1=$NHCOCH_3$

TABLE 2. Comparative Curaremimetic Activities of Melliktin, Lycaconitine, and Nudicauline

Compound	Doses(mg/kg or g/ml) causing				
	IHS in rabbits	NMTP in cats	NMTP of a rat phrenic-diaphragm preparation	Suppression of the ACh contraction of the frog *musculus rectus abdominis*	Suppression of the amplitude of evoked and miniature end-plate potentials
Melliktin	3.0	2.2 (2+2.5)	$2 \cdot 10^{-5}$	$2 \cdot 10^{-5}$	$1 \cdot 10^{-6}$
Lycaconitine	2.0	1.4 (1.25+1.7)	$9 \cdot 10^{-6}$	$1 \cdot 10^{-5}$	$4 \cdot 10^{-7}$
Nudicauline	1.25	1.1 (0.8+1.2)	$5 \cdot 10^{-6}$	$7 \cdot 10^{-6}$	$1 \cdot 10^{-7}$

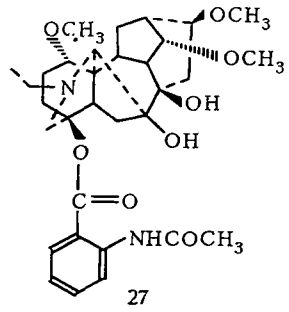
— the stereoelectronic situation at the nitrogen atom also affects the degree of activity. The introduction of an amide carbonyl group substantially lowers curaremimetic activity. 19-Oxolycoctonine (**26**), obtained by the method of [24], is an order of magnitude less active than the initial alkaloid.

The degree of curaremimetic action of the lycoctonine alkaloids depends on the system of substituents definitely located in space. The most preferential for the manifestation of curaremimetic activity is the presence of C-1αOH, C-14αOH, and C-18OAr. The nature of the substituents in these positions is of great importance for the manifestation of activity. With a successive change in the substituents at C-1, muscle relaxant activity decreases in the sequence OH < OCH_3 < OAc < OAr. Conversely, the analogous replacement of a hydroxy group at C-14 and at C-18 by methoxy, acetoxy, and aroyloxy groups leads to an enhancement of curaremimetic activity.

Mention must be made of the great similarity in chemical structure of the alkaloids anthranoyllycoctonine (**18**), ajacine (**24**), isolappaconitine (**27**) and N-deacetyllappaconitine (**28**). However, in spite of their close similarity in chemical structure, these substances possess qualitatively different pharmacological properties, which is obviously connected with the presence of a C-18 methylene group in alkaloids (**18**) and (**24**).

While the alkaloids anthranoyllycoctonine and ajacine possessed pronounced hypotensive, ganglion-blocking, and curaremimetic effects and at the same time exerted no appreciable influence on sensitive nerve terminals, the smooth musculature, and the functions of the cardiac conductor system, the alkaloids N-deacetyllappaconitine and isolappaconitine did not exhibit hypotensive or pronounced peripheral H-cholinolytic action and they suppressed the excitability and conductivity of the cardiac tissue and produced antiarrhythmic, spasmolytic, local anesthetic, analgesic and antinflammatory effects.

By making use of the laws revealed it is possible to construct new drugs with a curaremimetic action. Correspondingly, the pharmacophore for curaremimetic drugs can be represented by formula (**29**).



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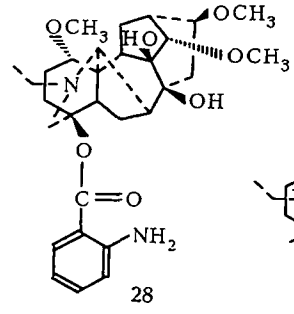

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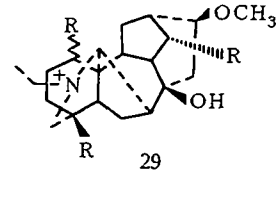

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PHARMACOLOGICAL INVESTIGATION OF THE ALKALOIDS NUDICAULINE AND LYCOCONITINE IN COMPARISON WITH MELLIKTIN

The alkaloid nudicauline has been isolated from the plant *Delphinium elatum* cultivated in the Polar-Alpine Botanical Garden [17], and lycaconitine from *Aconitum umbrosum* [16]. Both species undergo cultivation well. The levels of nudicauline and lycaconitine in the plants range from 2 to 3% of the dry mass of raw material. In chemical structure lycaconitine is close to methyllycaconitine, and nudicauline combines within itself elements of structural similarity to methylycaconitine and condelphine.

The curaremimetic activities and action mechanisms of nudicauline and lycaconitine in the form of perchlorates were investigated *in vitro* on isolated organs and *in vivo* on mice, rabbits, cats, and dogs. In experiments on intact mice and rabbits, nudicauline and lycaconitine caused similar well-defined muscle-relaxing effects with various methods of administration: intravenously, intraperitoneally, and intragastrically. Under these conditions they were superior to melliktin in muscle-relaxing activity. With respect to acute toxicity in mice, lycaconitine and nudicauline were somewhat more active than melliktin (see Table 2).

The initial symptoms of neuromuscular relaxation on the intravenous injection of nudicauline and lycaconitie into rabbits appeared at doses of 0.75-1 mg/kg. The action of melliktin began to appear at a dose of 1.5 mg/kg. In rabbits, the IHS set in on the administration of 2 mg/kg of lycaconitine, 1.25 mg/kg of nudicauline, and 3 mg/kg of melliktin. No fasciculation was observed here. The IHS was usually accompanied by relaxation of the muscles of the extremities and by dyspnea.

On an isolated rat phrenic-diaphragm preparation melliktin and lycaconitine in concentrations of $2 \cdot 10^{-6}$-10^{-4} g/ml blocked the neuromuscular transmission of impulses by 20-100%. Lycaconitine revealed a higher activity than melliktin. The concentration causing a 50% decrease in the amplitude of the contraction of the diaphragm muscle was $2 \cdot 10^{-5}$ g/ml for melliktin and $9 \cdot 10^{-6}$ g/ml for lycaconitine.

In experiments on anesthetized cats and dogs, nudicauline in doses of 0.8-1.2 mg/kg and lycaconitine in doses of 1.25-1.5 mg/kg led to the complete blockage of nerve–muscle conductivity for 28-35 min. Under similar experimental conditions, melliktin in doses of 1-1.5 mg/kg suppressed neuromuscular transmission by 20-70%, and in doses of 2-2.5 mg/kg caused complete transmission block.

It follows from Table 2 that the curaremimetic activities of lycaconitine and nudicauline are 1.5-2 times greater than that of the melliktin currently used in practical medicine.

In the mechanism of the muscle-relaxing action, lycaconitine and nudicauline, like melliktin, belong to the antidepolarizing muscle relaxants. This is shown by the following facts:

— the intravenous injection of proserine rapidly restored the neuromuscular transmission suppressed by these alkaloids; and,

— in *in vitro* experiments these compounds did not affect tonus but suppressed the contraction of frog *musculus rectus abdominis* caused by acetylcholine (10^{-6} g/ml).

The method of an intracellular lead of membrane potentials in an isolated frog *musculus sartorius* showed that nudicauline, lycaconitine, and melliktin do not affect the membrane potential of the muscle fiber and the frequency of spontaneous miniature potentials, which shows the absence of a presynaptic and direct depolarizing action.

The compounds lowered the amplitude of the evoked and miniature end-plate potentials and led to the complete blockage of the acetylcholine receptors of the postsynaptic membrane. In the strength and rate of onset of neuromuscular block, nudicauline and lycaconitine were superior to melliktin (see Table 2).

As the comparative investigations showed, nudicauline and lycaconitine, like melliktin, exert a depressing effect on sympathetic and parasympathetic ganglia and cause hypotension.

The compounds exerted no appreciable influence on M-cholinoreceptors, adrenoreceptors, and histamine receptors and did not affect the frequency of cardiac contractions, the functions of the conductive system of the heart, and the tonus and spontaneous activity of the small intestine and the peripheral vessels, but in large doses they showed a weak central H-cholinolytic action.

Among the alkaloids studied, karakoline, 14-acetylvirescenine, lycaconitine, and nudicauline have been proposed as bioreagents for fundamental and applied medicobiological investigations and are being produced by the French firm LATOXAN.

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