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DITERPENOID ALKALOIDS AS A NEW CLASS OF ANTIARRHYTHMIC AGENTS. STRUCTURE–ACTIVITY RELATIONSHIP

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The cardiotropic activities of 111 diterpene alkaloids and their synthetic derivatives having lycoctine, heteratisine, napelline, and denudatine skeletons have been studied. It has been established that the overwhelming majority of these compounds exhibit a pronounced antiarrhythmic and antifibrillatory action, which is a finding of practical interest. Their structure–activity relationships – the directions of their action in relation to the results of x-ray structural analysis – have been investigated. The following have been selected and proposed as antiarrhythmic agents for practical medicine: lappaconitine, deacetyllappaconitine, 6-benzoylheteratisine, 14-benzoyl talatisamine, 1-benzoylnapelline, and others. Of them, lappaconitine hydrobromide (allapinin) has been introduced into public health practice.

In the present paper we give the results of complex investigations over many years directed to a search for drugs with a cardiorhythmic action among various structural types of diterpene alkaloids (DAs) isolated from plants of the *Aconitum* and *Delphinium* genera [1, 2], and their synthetic derivatives. The starting point was the assumption that among structurally close analogs of arrhythmogenic alkaloids of the aconitine type substances might be found that exhibit a pronounced antiarrhythmic action. The antiarrhythmic properties detected in napelline and heteratisine [3] induced us to undertake broad screening among various structural types of DAs, to form ideas on structure–activity relationships, and to make planned modifications with the aim of obtaining highly active drugs having a cardiorhythmic action.

CARDIORHYTHMIC ACTION AND STRUCTURE–ACTIVITY RELATIONSHIPS OF DAS WITH THE LYCOCTONINE SKELETON

We investigated 82 DAs and their derivatives of the above type, which, from the nature of their substituents, may be divided into three types: 1) amino alcohols; 2) amino alcohol acetates; and 3) O-aroyl-substituted amino alcohols.

The screening of the substances for antiarrhythmic activity was conducted on models of aconitine arrhythmia in anesthetized rats and of irreversible cardiac fibrillation in alert mice. The rats were injected with the preparations intravenously in doses rising to 1/10 of their LD_{50} 3-5 min before the intravenous administration of aconitine in a dose of 10-12 μg/kg, and the mice intraperitoneally 25-30 min before the intravenous administration of an absolutely lethal dose of aconitine – 200 μg/kg (LD_{100}). The antiarrhythmic activities of the compounds were compared with that of novokainamid [procaine amide hydrochloride]. As a criterion of the breadth of the antiarrhythmic and (or) antifibrillatory action we took the ratios LD_{50}/ED_{50} which we called the antiarrhythmic and antifibrillatory indices (AAI and AFI).

The investigations performed established that, with respect to the nature of their action on cardiac rhythm, the compounds of the series studied possess two qualitatively different types of pharmacological action: I) arrhythmogenic – aconitine-like (neurotoxic); and II) antiarrhythmic – quinidine-like.

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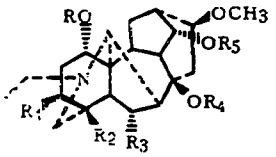
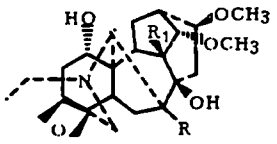
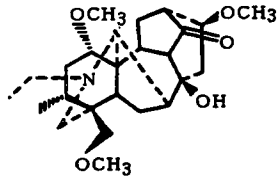


1. R_2=CH_3 (Here and below, if R is not specified it is H)
2. R=$COCH_3$;R_2=CH_3
3. R=COC_6H_5;R_2=CH_3
4. R=COC_6H_5;R_2=CH_3; R_5=$COCH_3$
5. R=$COCH_3$;R_2=CH_3;R_5=COC_6H_5
9. R_2=OH;R_5=CH_3
11. R=$COCH_3$;R_1=$OCOCH_3$;R_2=Cl; R_5=CH_3
12. R_1=$OCOC_6H_5$;R_2=Cl;R_5=CH_3
25. R=R_5=CH_3; R_2=$OCOC_6H_4NHCOCH_3$
44. R_2=CH_2OCH_3;R_3=OCH_3
47. R_2=CH_2OCH_3
48. R_2=CH_2OCH_3;R_5=$COCH_3$
49. R=COC_6H_5;R_2=CH_2OCH_3; R_5=$COCH_3$
50. R=COC_6H_5;R_2=CH_2OCH_3
51. R=CH_3;R_2=CH_2OCH_3
53. R=CH_3;R_2=CH_2OCH_3; R_5=$COCH_3$
54. R=CH_3;R_2=CH_2OCH_3;R_4=R_5=$COCH_3$
55. R=CH_3;R_2=CH_2OCH_3; R_5=COC_6H_5
56. R=CH_3;R_2=CH_2OCH_3;R_4=$COCH_3$; R_5=COC_6H_5



6.
7. R=OH
10. R_1=OH



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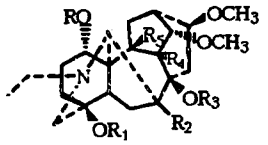

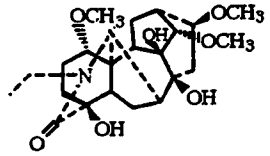

8. R_2=OH
13. R=CH_3;R_4=OH
15. R_4=OH
16. R=R_3=CH_3;R_4=OCH_3
17. R=CH_3;R_1=R_3=$COCH_3$;R_4=$OCOCH_3$
18. R=CH_3;R_1=COC_6H_4-2-$NHCOCH_3$;R_4=OH
19. R=CH_3;R_1=COC_6H_4-2-NH_2;R_4=OH
20. R=CH_3;R_1=COC_6H_4-2-$NHCOCH_3$;R_3=$COCH_3$;R_4=$OCOCH_3$
21. R=R_3=CH_3;R_1=COC_6H_4-2-$NHCOCH_3$;R_4=OCH_3
22. R=CH_3;R_1=COC_6H_4-2-NH_2;R_4=R_5=OH
23. R=CH_3;R_1=COC_6H_4-2-$NHCOCH_3$;R_2=R_4=OH
24. R=CH_3;R_1=COC_6H_4-2-$NHCOCH_3$;R_2=OH
26. R=CH_3;R_1=COC_6H_4-2-$NHCOCH_3$;R_4=R_5=OH

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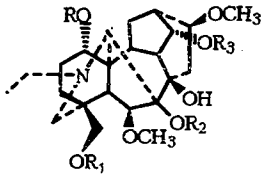
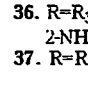
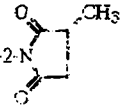


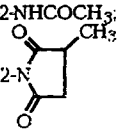
27. $R=R_3=CH_3$
28. $R=R_1=R_3=CH_3$
29. $R=R_1=R_2=R_3=CH_3$
30. $R=CH_3$
31. $R_3=CH_3$
32. $R=R_3=CH_3;R_1=COC_6H_4\text{-}2\text{-}NH_2$
35. $R=R_3=CH_3;R_1=COC_6H_4\text{-}2\text{-}NHCO(CH_2)_2COOH$
36. $R=R_3=CH_3;R_1=COC_6H_4\text{-}2\text{-}NHCOCH_3$
37. $R=R_3=CH_3;R_1=COC_6H_4\text{-}2\text{-}N$



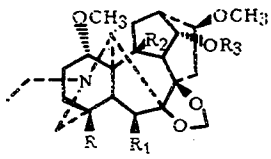
38. $R=R_3=CH_3;R_1=COC_6H_4\text{-}2\text{-}N$

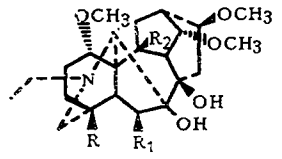


39. $R=CH_3;R_1=COC_6H_4\text{-}2\text{-}NH_2$
40. $R=CH_3;R_1=COC_6H_4\text{-}2\text{-}NH_2;$ $R_3=COCH_3$
41. $R=CH_3;R_1=COC_6H_4\text{-}2\text{-}NHCOCH_3;$
42. $R=CH_3;R_1=COC_6H_4\text{-}2\text{-}NHCOCH_3;$ $R_3=COCH_3$
43. $R=CH_3;R_1=COC_6H_4\text{-}2\text{-}N$ $R_3=COCH_3$

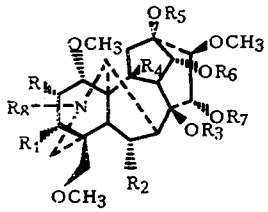


45. $R_1=R_3=CH_3$
46. $R_1=R_2=R_3=CH_3$
57. $R=R_1=CH_3$
58. $R=R_1=CH_3;R_3=COCH_3$
59. $R=R_1=CH_3;R_3=COC_6H_5$
60. $R_1=CH_3$
61. $R_1=CH_3;R_3=COC_6H_5$





62. $R=CH_2OCH_3;R_1=OH;$ $R_3=CH_3$
63. $R=CH_2OCH_3;R_3=CH_3$
64. $R=CH_2OCH_3;R_1=OH$
65. $R=R_3=CH_3;R_1=R_2=OH$
66. $R=CH_3;R_1=R_2=OH$
67. $R=R_3=CH_3;R_1=OCOCH_3;$ $R_2=OH$
68. $R=CH_2OCH_3;R_1=OCOC_6H_5;$ $R_3=CH_3$
69. $R=R_3=CH_3;R_1=OCOC_6H_5;$ $R_2=OH$
70. $R=CH_3;R_1=OCOCH_3;R_2=OH;$ $R_3=COC_6H_5$



71. $R_1=OH;R_2=OCH_3;R_8=C_2H_5$
72. $R_1=OH;R_2=OCH_3;R_6=COC_6H_5;R_8=C_2H_5$
73. $R_1=OCOCH_3;R_2=OCH_3;R_3=R_5=R_7=COCH_3;R_6=COC_6H_5;R_8=C_2H_5$
74. $R_1=OH;R_2=OCH_3;R_3=COCH_3;R_6=COC_6H_5;R_8=C_2H_5$
75. $R_1=R_4=OH;R_2=OCH_3;R_3=COCH_3;R_6=COC_6H_5;R_8=C_2H_5$
76. $R=R_1=OH;R_2=OCH_3;R_3=COCH_3;R_6=COC_6H_5;R_8=C_2H_5$
77. $R_1=OH;R_2=OCH_3;R_3=COCH_3;R_6=COC_6H_5;R_8=CH_3$
78. $R_1=OH;R_2=OCH_3;R_3=COCH_3;R_6=COC_6H_5;R_8=H$
79. $R_2=OCH_3;R_3=COCH_3;R_6=COC_6H_5;R_8=CH_3$
80. $R_1=OCOCH_3;R_2=OCH_3;R_3=COCH_3;R_6=COC_6H_5;R_8=C_2H_5$
81. $R_1=OCOCH_3;R_2=OCH_3;R_3=R_7=COCH_3;R_6=COC_6H_5;R_8=C_2H_5$
82. $R_1=OCOC_6H_5;R_2=OCH_3;R_3=COCH_3;R_6=R_7=COC_6H_5;R_8=C_2H_5$

Among the substances studied (Table 1), an arrhythmogenic action was shown by nine compounds (**74-82**), the most active of these being the alkaloids mesaconitine, noraconitine, and aconitine. In their effect on the main functions of the heart (excitability, conductivity, contractility), all the other DAs exhibited the combination of properties characteristic for drugs with an antiarrhythmic action. In addition, the compounds assigned to group II showed a pronounced antagonism to the substances included in group I, preventing and (or) curing cardiac arrhythmia and fibrillation in rats and mice. The comparative antiarrhythmic efficacies of the compounds tested and of novokainamid on model aconitine arrhythmia in rats and on cardiac fibrillation in alert mice are given in Table 1.

TABLE 1. Comparative Toxicities and Antiarrhythmic Activities of DAs and Their Derivatives with a Lycoctonine Skeleton on Models of Aconitine Arrhythmia in Anesthetized Rats and of Lethal Cardiac Fibrillation in Alert Mice

Compound	Antiarrhythmic activity in experiments on rats (aconitine, 10-12 μg/kg)			Antifibrillatory activity in mice (aconitine, 200 μg/kg)		
	intravenously, mg/kg			intraperitoneally, mg/kg		
	LD_{50}	ED_{50}	AAI, LD_{50}/ED_{50}	LD_{50}	ED_{50}	AFI, LD_{50}/ED_{50}
1. Karakoline	51.5	16.5	3.1	300	Ineffective in doses of 50-200 mg/kg	
2. 1-Acetylkarakoline	125.3	14.0	8.9	–		
3. 1-Benzoylkarakoline	21.8	3.0	7.3	130	22	5.9
4. 14-Acetyl-1-benzoyl-karakoline	36.3	1.8	20.2	135	25.5	5.3
5. 1-Acetyl-14-benzoyl-karakoline	18.5	0.46	40.2	–	–	–
6. Monticamine	250	40	6.3	735	Ineffective in doses of 50-200 mg/kg	
7. Monticoline	495	20	24.7	>1000		
8. Dihydromonticoline	430	22.1	19.4	>1000	Ineffective in doses of 50-200 mg/kg	
9. Dihydromonticamine	220	Ineffective in doses of 20-40 mg/kg		>800		
10. Excelsine	130	28	4.6	–	"	
11. 1,3-Diacetyl-4-chloro-monticamine	78	Ineffective in doses of 10-40 mg/kg		–	Ineffective in doses of 50-200 mg/kg	
12. 3-Benzoyl-4-chloro-monticamine	29	Ineffective in doses of 10-20 mg/kg		–	Ineffective in doses of 50-200 mg/kg	
13. Lappaconine	195	22	8.9	400	200	2
14. Oxolappaconine	2900	Ineffective in doses of 20-100 mg/kg		–	Ineffective in doses of 1000-2000 mg/kg	
15. Lappaconidine	195	18	10.8	400	100	4
16. Dimethyllappaconine	230	Ineffective in doses of 20-50 mg/kg		–	Ineffective in doses of 100-200 mg/kg	
17. 4,8,9-Triacetyl-lappaconine	145	13	11.2	300		–
18. Lappaconitine	5.9	0.05	118	15.5	0.48	32.3
19. N-Deacetyl-lappaconitine	7.3	0.05	146	35	0.8	43.8
20. Lappaconitine 8,9-diacetate	25.9	2	13	–	–	–
21. 8,9-Dimethyl-lappaconitine	205	6.5	31.5	–	–	–
22. Sepaconitine	16.5	0.21	79	62.2	23	2.7
23. Ranaconitine	6.2	0.05	124			
24. Isolappaconitine	–	0.25	–	–	–	–
25. 9-Deoxylappaconitine	–	0.2	–	–	–	–
26. N-Acetylsepaconitine	15	0.07	214.3	–	–	–
27. Lycoctonine	170	Ineffective in doses of 10-40 mg/kg		–	Ineffective in doses of 50-200 mg/kg	
28. Delphatine	100	40.1	2.5	600	100*	6
29. Methyldelphatine	76.5	25*	3.1	286	70*	4.1
30. Delectinine	130	Ineffective in doses of 10-30 mg/kg		–	Ineffective in doses of 50-200 mg/kg	
31. Gigactonine	88.0			–		
32. Anthranoyllycoctonine	20.1	Ineffective in doses of 1-10 mg/kg		–	Ineffective in doses of 10-50 mg/kg	
33. Leucophine				–		
34. Leuconine						
35. Puberaconitine	22.5	Ineffective in doses of 1-10 mg/kg			Ineffective in doses of 5-25 mg/kg	
36. Ajacine	9.0			35.4		
37. Lycaconitine	2.6	"		12.5	"	
38. Methyllycaconitine	3.9	"		19.5	"	
39. Delectine	35.8	"		>100	"	
40. O-Acetyldelectine	15.5	"		>50	"	
41. N-Acetyldelectine	25.3	"		>100	"	
42. N,O-Diactyldelectine	12.5	"		>50	"	
43. Nudicauline	1.8	"		13.0	"	
44. Neoline	69	Ineffective in doses of 5-20 mg/kg		244.5	Ineffective in doses of 50-200 mg/kg	
45. Delcoline	175			550		
46. Methyldelcoline	97.5	"		–		
47. Isotalatisidine	40.1	Ineffective in doses of 5-20 mg/kg		170	Ineffective in doses of 25-100 mg/kg	
48. Condelphine	18.5	3*	6.2	–		
49. 1-Benzoylcondelphine	54.1	1.1	49.2	250	39	6.4
50. 1-Benzoylisotalatisidine	25.3	2.6	9.7	120	28	4.3

TABLE 1 (continued)

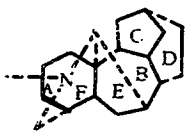
Compound	Antiarrhythmic activity in experiments on rats (aconitine, 10-12 μg/kg) intravenously, mg/kg			Antifibrillatory activity in mice (aconitine, 200 μg/kg) intraperitoneally, mg/kg		
	LD_{50}	ED_{50}	AAI, LD_{50}/ED_{50}	LD_{50}	ED_{50}	AFI, LD_{50}/ED_{50}
51. Talatisamine	110.0	18x	6.1	>300	100	>3
52. Dehydrotalatisamine	92.5	15x	6.2	>300	75	>4
53. 14-Acetyltalatisamine	70.7	10x	7.0	–	–	–
54. 8,14-Diacetyl-talatisamine	24.0	3x	8.0	–	–	–
55. 14-Benzoyl talatisamine	25.0	0.26	96.2	122.5	6.6	18.5
56. 8-Acetyl-14-benzoyl-talatisamine	15.0	0.8	18.7	130	8.0	16.2
57. Browniine	70	Ineffective in doses of 5-20 mg/kg		450	Ineffective in doses of 50-200 mg/kg	
58. 14-Acetylbrowniine	57	10x	5.7	300	"	
59. 14-Benzoylbrowniine	17.5	0.66	26.5	125	7.5	16.7
60. Delcosine (iliensine)	108.7	Ineffective in doses of 5-20 mg/kg		>200	Ineffective in doses of 50-200 mg/kg	
61. 14-Benzoyldelcosine	35.1	1.8	19.5	>100	20	>5
62. Delcorine	116	15	7.7	590	170	3.5
63. Deoxydelcorine	46.5	10	4.7	235	74	3.2
64. Demethylenedelcorine	120	20	6	>500	100x	>5
65. Eldelidine	235	25.4	9.3	>500	200x	>2.5
66. Demethyleneeldelidine	230	30	7.8	>500	200x	>2.5
67. Eldeline	136	10.2	12.7	>500	200x	>2.5
68. 6-Benzoyldelcorine	45	2	22.5	205	–	–
69. 6-Benzoyleldelidine	16.1	0.67	24	66	10	6.6
70. 14-Benzoyldicty-ocarpine	22.1	0.82	27	100	30	3
71. Aconine	200	60	2.7	>450	100x	>4.5x
72. Benzoylaconine	16	1x	16	>50	15	>3
73. 3,13,15-Triacetyl-aconitine	150	5.5	27	>500	60	>3
ARRHYTHMOGENIC ALKALOIDS OF THE TYPE OF ACONITINE						
	Doses causing cardiac arrhythmia in anesthetized rats on a single intravenous administration			Doses causing fatal cardiac fibrillation and death of 50% of mice (LD_{50}) on intravenous administration		
74. Aconitine	0.01			0.125		
75. Aconifine	0.011			0.22		
76. Altaconitine	0.2			2.2		
77. Mesaconitine	0.003			0.085		
78. Noraconitine	0.005			0.15		
79. Hypaconitine	0.015	"		0.16		
80. 3-Monoacetyl-aconitine	0.25	"		0.27	"	
81. 3,15-Diacetyl-aconitine	0.3	"		3.5	"	
82. 3,15-Dibenzoyl-aconitine	3.0	"		13.2	"	
NOVOKAINAMID [procaine amide hydrochloride]	138	60	2.3	445	Ineffective in doses of 50-200 μg/kg	

Note. (ED_{50}). The dose preventing the phenomenon of cardiac arrhythmia or fibrillation in 50% of animals; x) the dose causing a 5-fold increase in the latent period of aconitine arrhythmia; o) the absence of an antiarrhythmic effect.

Antiarrhythmic activity is characteristic both of amino alcohols and of their esters. As can be seen from Table 1, the antiarrhythmic activity of DAs rises in the sequence amino alcohols < monoacetyl derivatives of amino alcohols < monoaromatic derivatives of amino alcohols. Furthermore, together with the rise in antiarrhythmic activity, there is a rise in cardioselectivity.

Among the compounds investigated, the most pronounced antiarrhythmic and antifibrillatory action was shown by the alkaloids (**18**), (**19**), (**22**), (**23**), (**24**), (**25**), (**26**), and (**55**). These compounds were more than 1000 times superior in antiarrhythmic activity and more than 50-fold superior in breadth of therapeutic action to the drug novokainamid currently used in practical medicine. Unlike novokainamid, the DAs exerted a powerful protective antifibrillatory action and prevented the death of animals poisoned with a lethal dose of aconitine.

The carbon skeleton of lycoctonine (*A*) is a hexacyclic structure consisting of four six-membered (*A, B, D,* and *F*) and two five-membered (*C* and *E*) rings.

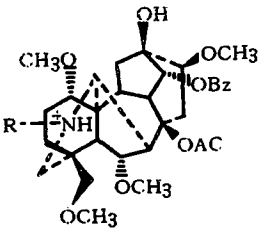


A

According to the Cambridge Crystallographic Data Centre, at the present time about 40 compounds with the lycoctonine skeleton have been studied by x-ray structural analysis (XSA). The structural diversity of these alkaloids is determined by the number, positions, natures, and stereochemistries of the oxygen substituents. The absolute configuration, which is determined by the linkage of the rings, remains unchanged for all cases. On the basis of the XSA results, NMR spectroscopy, and a consideration of molecular models, limited conformational isomerism is possible for the lycoconine skeleton. In it, rings *A* and *F* are labile. Ring *A* can assume various forms, depending on the natures and positions of the substituents and the conditions of its environment. However, according to the XSA results, in crystals of salts with protonated nitrogen (which are used in pharmacological investigations), ring *A* stably adopts the 2,5α-boat conformation, regardless of the nature of the substituent at C1 (see, for example [4-10]). In alkaloids with the lycoctonine skeleton the six-membered ring *B* is present mainly in the 11α,17β-half-chair conformation, although, depending on its chemical environment, it may pass into close forms – an 11α- or a 17β-envelope. In all lycoctonine alkaloids the conformations of rings *C, D,* and *E* are retained, regardless of the presence and positions of substituents; however, slight distortions of them from the canonical form are observed.

Thus, the system of rings *B, C, D,* and *E* of the lycoctonine skeleton is a rigid section, while rings *A* and *F*, which are subject to conformational changes, and groupings mobile through rotation about C–O bonds (OBz, OAc, OMe, etc.) may play the role of a flexible section undergoing rearrangement in the presence of the receptor.

Analysis shows that the qualitative directivity of the cardiorhythmic action of the alkaloids is due to the system of substituents. In spite of the difference in the number and positions of the substituents among the nine alkaloids with an aconitine-like action (see Table 1), the presence of the pharmacophore B is a common feature of them [11, 12].



B

The length of the carbon chain at N (–H, $-CH_3$, $-C_2H_5$), the nature of the aromatic grouping at C14 (O–Ar), and the number of additional OH groups and their positions affect the degree of the neurotoxic effect but do not lead to the appearance of qualitatively new properties for the compounds. Consequently, the arrhythmogenic action of substances of the aconitine type is due to the presence in their molecules, together with other common elements of their structures, of the triad of substituents consisting of C13β–OH, C14α–OAr, and C8β–OAc, and a positively charged nitrogen atom.

The alkaloid aconitine is widely used in various types of medicobiological investigations as a fine neurochemical tool for modeling arrhythmias, studying the mechanisms of their appearance, and investigating the processes forming the basis of the generation of a nervous impulse. Figure 1 shows a fragment of the chemical structure of a typical representative of the first

TABLE 2. Comparative Efficacies of Compounds of the Heteratisine Type and Known Antiarrhythmic Drugs in the Aconitine-induced Arrhythmia of Rats

Compound	LD_{50}	ED_{50}	LD_{50}/ED_{50}
83. Dehydroheteratisine	165.0	20.0	8.2
84. Heteratisine	180.0	12.5	14.4
85. Acetylheteratisine	182.0	5.0	36.5
86. Benzoylheteratisine	5.0	0.035	142.9
87. Furoylheteratisine	16.2	0.07	231.4
88. Anisoylheteratisine	6.0	0.05	120.0
89. Veratroylheteratisine	42.5	2.0	21.2
90. Phenylacetylheteratisine	60.2	1.8	33.4
91. *p*-Nitrobenzoylheteratisine	27.5	0.2	137.5
Ritmilen	42.0	4.0	10.5
Étmozin [ethmosine]	12.0	1.25	9.6

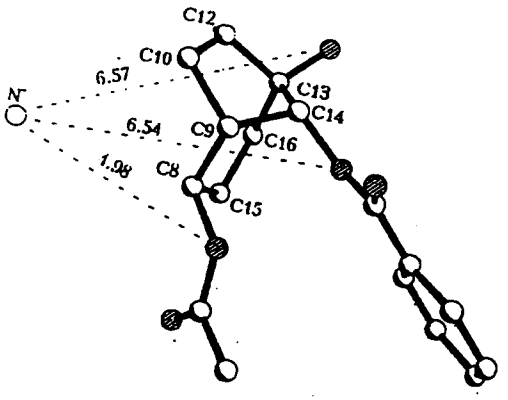


Fig. 1

group – the alkaloid aconitine. The triad of substituents responsible for the cardiotonic effect has been isolated and shown in space. The distances in Angströms between the positively charged nitrogen atom and these substituents are given.

If we start from the generally accepted assumption that the presence and mutual positions of chemical groups and bonds in the molecule of a substance must correspond in complementary fashion to definite chemical groups of the receptors, it is necessary to assume the presence in the active center of the receptor of at least four points at which it interacts with the most functionally important groups of these compounds. Taking into account the rigidity of the lycoctonine skeleton, which ensures constancy of interatomic distances and stereochemistry, it is not difficult to draw up a hypothetical model of the active center of the toxin receptor. It may be assumed that the active center of the receptor contains:

1) an anionic group bearing a negative charge and capable of binding by electrostatic forces with the positively charged nitrogen atom;

2) two hydrophobic groups forming intermolecular bonds with the acetic acid and aromatic acid residues; and

3) a group capable of forming a hydrogen bond with the C13α–OH.

Destruction of the integrity of this system, consisting of three substituents, leads to a loss of the specific arrhythmogenic effect of aconitine and the acquisition of qualitatively opposite properties. The DAs studied that did not contain in their structure the substituent system characteristic of aconitine have a qualitatively different type of cardiorhythmic activity. The majority of them possess a pronounced antiarrhythmic action and exhibit antagonism to the arrhythmic action of acontine.

An analysis of chemical structure and antiarrhythmic activity reveals common features characteristic for the series of compounds studied. Functionally, the C1, C4, C6, and C14 positions and the basicity of the nitrogen atom are most important for the manifestation of antiarrhythmic activity. A decrease in the basicity of the nitrogen atom in the lycoctonine skeleton leads to a sharp fall in antiarrhythmic activity (oxolappaconine is considerably less active than lappaconine). Amino alcohols containing substituents of simple nature in their molecules exhibit a relatively weak antiarrhythmic activity.

In a number of cases, the acetylation of amino alcohols leads to a rise in antiarrhythmic activity and an increase in cardioselectivity. However, this increase is relatively small. No direct relationship has been detected between antiarrhythmic activity and the number of ester groups in a series of mono-, di-, and triacetyl derivatives of amino alcohols.

The introduction of an aromatic ester substituent into one of the C1, C4, C6, and C14 positions of the lycoctonine skeleton leads to a sharp rise in antiarrhythmic activity and in the selectivity of the effect, and the basic possibility has been

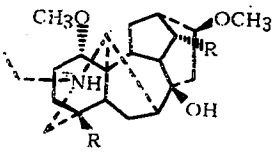
shown of creating compounds with a high antiarrhythmic activity from inactive amino alcohols and their acetates. For example, the alkaloid condelphine possesses no antiarrhythmic action, while 1-benzoylcondelphine is 54 times superior to novokainamid in antiarrhythmic activity. When substituents of different natures are present at C1, C4, C6, or C14, on the whole the antiarrhythmic activity of the compounds rises in the sequence amino alcohols – amino alcohol acetates – monoaromatic derivatives of amino alcohols. When an aromatic substituent is present in the molecule of an alkaloid, the additional introduction of an acetyl group may lead to a weakening, an enhancement, or a qualitative change in the direction of the action of the compound.

Hydroxy groups are capable of exerting a considerable influence on antiarrhythmic activity. Depending on the position of a hydroxy group and its relationship to other substituents, its effect may be dual – in some cases an enhancement, and in others a weakening, of antiarrhythmic activity is observed. For example, when substituents of different natures (OH, OMe, OAc, OBz) are present in the C14 position, a hydroxyl at C1 weakens the activities of the compounds, while replacement of the C1 hydroxy group by methoxyl leads to an enhancement of the antiarrhythmic activity of the same series of compounds.

The most active are the bisnorditerpenoid alkaloids such as lappaconitine (**18**). A common element of the structures of these compounds is the presence of a residue of acetylanthranilic or anthranilic acid at C4 and of methoxy groups at C1, C14, and C16 and a hydroxy group at C8. Differences consist in the presence of additional hydroxy groups at C7, C9, and C10. Saponification of an ester grouping (**18** → **13**) and the acetylation or methylation of free hydroxy groups (**18** → **20**, **18** → **21**) sharply lower the activity index.

Alkaloids of close chemical structure in which anthranilic acid is attached through a C18 methylene group (for example, 24 [sic] and **36**) exhibit no antiarrhythmic action. Characteristic for these compounds (**35** → **43**) are ganglioblocking and curaremimetic properties. Attention is attracted by the fact that such a fine structural difference as the presence of a C18 methylene group radically changes the direction of the pharmacological effect.

The second group of highly active antiarrhythmic agents is formed by alkaloids with a benzoyl group at C14. The most active among them is 14-benzoyltalatasamine (**55**), which has methoxy groups at C1, C16, and C18, and a hydroxyl at C8. Further complication of the molecule by the introduction of hydroxy groups in positions 6, 7, and 10, a methoxyl in position 6, and acetyl in position 6 considerably lowers the degree of antiarrhythmic action (compounds **58**, **59**, **61**, and **70**), 14-benzoyltalatasamine (**55**) is of interest for practical human and veterinary medicine, not only as a highly active antiarrhythmic agent but also as an effective antidote to aconitine, preventing the death of animals poisoned with an absolutely lethal dose of aconitine. On comparing the structural formulas of aconitine (**74**) and 14-benzoyltalatasamine (**55**) it is possible to see those functional groups (hydroxy groups at C3, C13, and C15, methoxyl at C6, and hydroxyl in place of acetyl at C8) the absence of which leads to a change of the highly toxic and arrhythmogenic properties into antiarrhythmic and antitoxic properties in relation to aconitine. Accordingly, the pharmacophore weakening the antiarrhythmic properties of the alkaloids under consideration may be represented by formula C, where one of the R's must be an O-aromatic substituent.



C

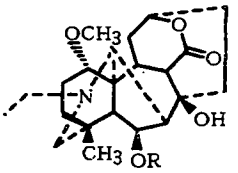
Derivatives of the lycoctonine alkaloids having aromatic ester substituents at C1 and C6 are considerably less active than those considered above. They are also characterized by common structural fragments: a monoaromatic ester substituent, a free hydroxy group, and oxygen functions at C14, and C16.

The general laws of the structure–activity relationship detected in the series of DAs with the lycoctonine skeleton are of great theoretical and practical value. On the basis of the results obtained it is possible with great probability to predict the existence of, and to carry out a directed search for, compounds with a high antiarrhythmic or arrhythmogenic-neurotoxic activity. It is most desirable to conduct the search for new antiarrhythmic drugs possessing a high activity and great breadth of therapeutic action in the C1, C4, C6, and C14 series of monoaromatic derivatives of alkaloids obtained by synthesis from amino alkaloids or isolated from plants. By changing the nature of the aromatic substituent in these positions and also the positions of the hydroxy groups in the molecule, it is possible to obtain a series of highly effective new drugs differing by the spectra and natures of their antiarrhythmic action.

INTERRELATIONSHIP BETWEEN STRUCTURE AND ANTI-ARRHYTHMIC ACTIVITY OF A SERIES OF DAS WITH THE HETERATISINE SKELETON AND THEIR ANALOGS

Using the same method and an analogous procedure of investigation, we have tested nine compounds with the heteratisine type of structure for antiarrhythmic activity. Table 2 gives the results on the antiarrhythmic efficacies of the compounds studied and of known antiarrhythmic drugs. These results show that compounds with the heteratisine skeleton are characterized by a pronounced antiarrhythmic action. In this series of compounds, the activity varies by a factor of more than 300, which shows that in compounds with the heteratisine skeleton the activity depends greatly on the nature of the substituent at C6 [13]. Among the compounds studied, the most powerful antiarrhythmic action was observed in substances with an aromatic acid residue at C6. Among the latter, the greatest activity was shown by 6-benzoylheteratisine and 6-furoylheteratisine. A comparison of the antiarrhythmic efficacies of benzoylheteratisine and furoylheteratisine with those of étmozin and ritmilen showed that the first two are superior by an order of magnitude in activity and breadth of therapeutic (antiarrhythmic) action to the most active antiarrhythmic drugs widely used in practical medicine.

According to the results of conformational calculations performed by the MMX86 program [14] using XSA results for heteratisine [15], in a series of DAs with the heteratisine skeleton and their analogs the carbon framework with fixed oxygen atoms, on the whole, retains its geometry, which is close to that of the lycoctonine alkaloids. However, as witnessed by the results of a comparison of the PMR spectra of compounds (**83-87**) and (**91**), the *C, D* ring system of the heteratisine skeleton, unlike that of lycoctonine, is subject to conformational changes.



84. R=H

85. R=$COCH_3$

86. R=COC_6H_5

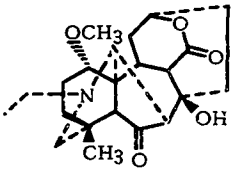
87. R=CO

88. R=COC_6H_4-4-OCH_3

89. R=COC_6H_3-3,4-$(OCH_3)_2$

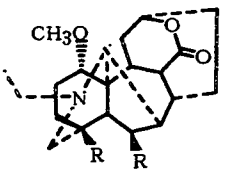
90. R=$COCH_2C_6H_5$

91. R=COC_6H_4-4-NO_2



83

In view of the presence of mobile oxygen substituents and a complex ring system in the molecules of both heteratisine and lycoctonine and the analogy between the interrelationship of chemical structure and antiarrhythmic action, we can with a certain degree of probability predict the desirability of a search for and (or) synthesis of new compounds with the heteratisine skeleton having the general formula D, where, in one of the cases shown, R may be an aromatic acid residue.



D

TABLE 3. Comparative Antiarrhythmic Activities of Diterpene Compounds with Napelline and Denudatine Skeletons and Novokainamid on Model Aconitine Arrhythmias in Rats and on Cardiac Fibrillation in Alert Mice

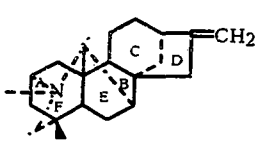
Compound	Antiarrhythmic activity in experiments on rats (aconitine – 10-12 μg/kg)			Antifibrillatory activity in mice (aconitine, 200 μg/kg)		
	intravenously, mg/kg			intraperitoneally, mg/kg		
	LD_{50}	ED_{50}	AAI, LD_{50}/ED_{50}	LD_{50}	ED_{50}	AFI, LD_{50}/ED_{50}
Alkaloids of the napelline type						
92. Songorine	142.5	7.3	19.4	480	25	19.4
93. Dihydrosongorine	120.0	12.0	10.0	450	28	15.3
94. Napelline	88.0	10.0	8.8	280	17.8	15.7
95. 12-Epinapelline	82.0	8.0	10.3	>250	20	>12.5
96. Songorine N-oxide	550.0	20.0	27.5	>2000	50–500 mg/kg	n.d.
97. Napelline N-oxide	725.0	28.0	25.8	>2000	50–500 mg/kg	n.d.
98. Songoramine	120.0	8.2	14.5	420		
99. 1-Acetylsongorine	150.0	15.0	10.0	420	50–100 mg/kg	n.d.
100. 1,15-Diacetylsongorine	131.0	18.0	7.3	805	50–100 mg/kg	n.d.
101. 12-Acetylnapelline	101.0	15.0	6.7	–	50.7	
102. 1-Acetylnapelline	100.0	15.0	6.7	310	–	
103. Songorine 12-semicarbazone	90.0	10.0	9.0	289	50–100 mg/kg	n.d.
104. Napelline 1-methacrylate	100.0	25.0	4.0	–	50–100 mg/kg	n.d.
105. Napelline 1-butyrate	66.0	20.0	3.3	–	50–100 mg/kg	n.d.
106. 1-Benzoylnapelline	30.0	0.24	133.3	135	3	45
107. 1-Benzoylsongorine	41.0	0.38	107.9	–	–	–
108. 1,12,15-Tribenzoylnapelline	175.0	20.0	8.8	–	50 mg/kg	n.d.
Alkaloids of the denudatine type						
109. Dictysine	155.0	15.0	10.3	–	46.5	
110. Dictysine acetonide	45.0	17.0	2.6	–	60.0	
111. Anhydrodemethanollappaconine	165.0	16.0	10.4	630	56.0	11.3
Novokainamid	138.0	60.0	2.3		50–200 mg/kg	n.d.

RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND ANTIARRHYTHMIC ACTION IN A SERIES OF DAS WITH NAPELLINE AND DENUDATLINE SKELETONS

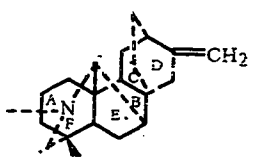
The spatial structure of compounds with napelline (E) and denudatine (F) structures is related to that of the lycoctonine alkaloids and consist of three six-membered (*A, C,* and *F*) and three five-membered (*B, D,* and *E*) rings. The denudatine skeleton, biogenetically linked with the atisine alkaloids, also consists of six rings, of which four (*A, C, D,* and *F*) are six- and two (*B* and *E*) are five-membered.

A characteristic feature of the denudatine and napelline skeletons, as of the lycoctonine skeleton, is the presence of a mobile 3-azabicyclo[3.3.1]nonane system, which, together with oxygen substituents in various positions plays the role of the flexible part of the mediator, the rigid section being the *B, C, E*-ring system.

We tested for antiarrhythmic activity 17 compounds of the napelline type and 3 compounds assigned, from their chemical structures, to the alkaloids of the denudatine type. The results of the experiments are given in Table 3. The majority



E



F

of the compounds studied exhibited a pronounced antiarrhythmic activity, exceeding in this respect the known antiarrhythmic drug novokainamid. Antiarrhythmic activity was shown by compounds of both types.

With the aim of finding highly active drugs, we have carried out the directed modification of napelline and songorine. Using a method of selective hydrolysis that we have developed, based on the difference in the rates of hydrolysis of ester groups of lycoctonine and napelline alkaloids [16], we synthesized the C1-acyl derivatives given in Table 3.

Of the 17 compounds belonging to the napelline type, the most pronounced antiarrhythmic and antifibrillatory actions were exhibited by 9 [sic] substances. With respect to the strength of their antiarrhythmic action they formed the following sequence: 1-benzoylnapelline (**106**), 1-benzoylsongorine (**107**), songorine (**92**) [sic], napelline (**94**), dihydrosongorine (**93**), 12-acetylnapelline (**102**), songorine N-oxide (**96**), and napelline N-oxide (**97**). The most active was 1-benzoylnapelline, which proved to be 166.6 times more active than novokainamid. Unlike novokainamid, in experiments on mice 1-benzoylnapelline exhibited a powerful antifibrillatory action and prevented the death of animals poisoned by the intravenous administration of a lethal dose of aconitine. 1-Benzoylnapelline was 41.6 times superior in antiarrhythmic activity to the initial napelline.

As can be seen from Table 3, in the antiarrhythmic activity on the two models of arrhythmia, compounds containing various aliphatic ester groups at C1, C12, and C15 were inferior to the initial compounds. (The antiarrhythmic activity falls in the sequence: songorine, napelline, songorine 12-semicarbazone, 12-acetylsongorine, 1-acetylsongorine, 1-acetylnapelline, 1,15-diacetylsongorine, napelline 1-butyrate, napelline 1-methacrylate.)

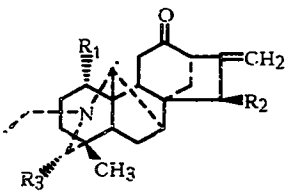
The specific antiarrhythmic activity of napelline falls sharply on the simultaneous benzoylation of the three hydroxy groups at C1, C12, and C15. In contrast to napelline, tribenzoylnapelline (**108**) in a dose of 20 mg/kg intravenously in rats and in doses of 50—100 mg/kg intraperitoneally in mice showed no protective antiarrhythmic action. Conversely, the antiarrhythmic activities of napelline and songorine rise sharply on the introduction of a monobenzoyl substituent into position 1.

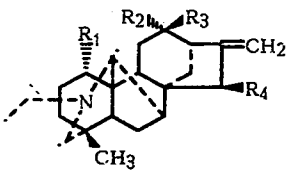
The results obtained show that the activities of this series of compounds change considerably according to the positions and natures of substituents at C1, C12, and C15. A monoaromatic ester substituent and free hydroxy groups are of great importance in the realization of the antiarrhythmic actions of songorine and napelline.

It is interesting to note that, in this series, N-oxides proved to be the least active and least toxic; however, even these showed a somewhat greater activity than novokainamid.

The marked difference in the antiarrhythmic activity and toxicity of songorine and napelline in comparison with their N-oxides shows the importance of the basicity of the nitrogen atom in the realization of the antiarrhythmic action of alkaloids of the napelline type.

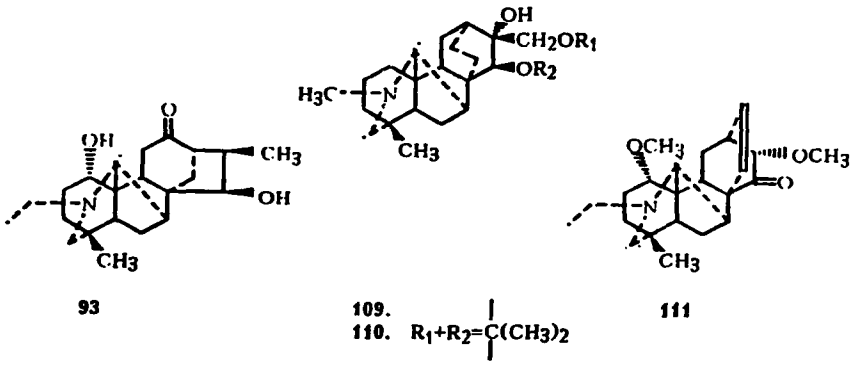
A moderate antiarrhythmic activity was shown by amino alcohols of the denudatine type: dictysine, dictysine acetonide, and anhydrodemethanollappaconine. In their antiarrhythmic activity, these compounds were 3-4 times better than novokainamid. Among this group of compounds, the greatest activity was shown by the alkaloid dictysine.





92. $R_1=R_2=OH$
96. $R_1=R_2=OH$; N→O
98. $R_1+R_3=-O-, R_2=OH$
99. $R_1=OCOCH_3; R_2=OH$
100. $R_1=R_2=OCOCH_3$
107. $R_1=OCOC_6H_5; R_2=OH$

94. $R_1=R_2=R_4=OH$
95. $R_1=R_3=R_4=OH$
97. $R_1=R_2=R_4=OH$; N→O
101. $R_1=R_4=OH; R_2=OCOCH_3$
102. $R_1=OCOCH_3; R_2=R_4=OH$
103. $R_1=R_4=OH; R_2+R_3=OCOCNNHCONH_2$
104. $R_1=OCO\text{-}C(=CH_2)\text{-}CH_3; R_2=R_4=OH$
105. $R_1=OCO(CH_2)_3CH_3; R_2=R_4=OH$
106. $R_1=OCOC_6H_5; R_2=R_4=OH$
108. $R_1=R_2=R_4=OCOC_6H_5$



93 109. 110. $R_1+R_2=C(CH_3)_2$ 111

Thus, the investigations performed have shown that alkaloids of the napelline and denudatine types possess a pronounced antiarrhythmic action. In antiarrhythmic efficacy, many of them are considerably superior to the antiarrhythmic drugs of the first group used at the present time. This shows the promising nature of the search for and creation of new antiarrhythmic drugs based on these compounds.

Among the compounds investigated, the most powerful antiarrhythmic and antifibrillatory activity was shown by 1-benzoylnapelline hydrochloride, which is distinguished from the antiarrhythmics described above by its comparatively low toxicity.

It is apparently desirable to conduct a further search for compounds with a high antiarrhythmic activity in this series of compounds by varying the position and nature of the ester substituent.

Thus, a new class of antiarrhythmic compounds of practical interest for medicine has been discovered. The relationship between chemical structure and cardiorhythmic action has been analyzed and structural-chemical features determining the arrhythmogenic and antiarrhythmic directions of a pharmacological action have been determined. Promising directions of the search for and the construction of new antiarrhythmic drugs have been noted.

A group of studies connected with the creation and introduction into medical practice of the most promising of the compounds found has been performed. Of them, lappaconitine hydrobromide (allapinin) has already been introduced into medical practice as an antiarrhythmic drug [17, 18]. Industrial methods have been developed for obtaining allapinin from the epigeal part of *Aconitum leucostomum* and the roots of *A. septentrionale*. 6-Benzoylheteratisine hydrochloride (benzerafin) is undergoing clinical trials. An industrial method for obtaining benzerafin from the epigeal part of *A. zeravschanicum* has been created. Work is being done on the introduction into medical practice of 1-benzoylnapelline hydrochloride [19] and 14-benzoyltalatasamine hydrochloride [20]. The two last-mentioned compounds may be used in human and veterinary medicine as antidotes in cases of the poisoning of people and animals by aconitine-like alkaloids.

The substances listed differ from one another by the speed of onset of the antiarrhythmic action and its duration and in their efficacy in relation to different forms of cardiac arrhythmia, which permits their use for treating a broad list of diseases.

Production of the alkaloid aconitine from the plant *A. soongoricum* has been organized [21]. New agonists and antagonists of aconitine have been proposed as bioreagents for various types of fundamental and applied investigations, and these are being placed on the world market by the French firm Latoxan.

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