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# ALKALOIDS OF *Aconitum coreanum*. X. CURARE-LIKE ACTIVITY—STRUCTURE RELATIONSHIP

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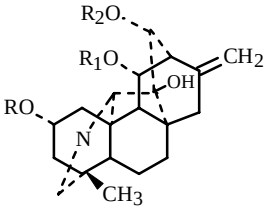
*The biological activity of 11 alkaloids isolated from* Aconitum coreanum *was studied. The compounds exhibit myorelaxant activity. Isoatisine and coryphine, which contain oxazolidine rings, are the most active. N-Oxides of 2-isobutyryl-14-hydroxyhetisine and 2-isobutyryl-13-acetyl-14-hydroxyhetisine are least active.*

**Key words:** *Aconitum coreanum*, Ranunculaceae, diterpenoid alkaloids, toxicity, curare-like activity.

*Aconitum coreanum* (Levl.) Rapaics (*A. komarovii* Steinb.) (Ranunculaceae) grows in the south of Primorskii Krai, in south-eastern China, and on the Korean peninsula [1]. It possesses antibacterial activity [2] and is poisonous [3]. Alkaloids have been detected in the roots, stems, leaves, and flowers [4]. The total alkaloids of this plant are highly toxic [5]. Roots of *A. coreanum* are used in China as a medicinal preparation [6].

A systematic investigation of *A. coreanum* growing near Chernyatino in Primorskii Krai has shown that it contains the diterpene alkaloids (DA) tangutisine (**1**) [7], acorine (**2**) [1], acoridine (**3**) [8], guan-fu-base Z (**4**) [1], guan-fu-base Z N-oxide (**5**) [9], guan-fu-base F (**6**) [10], guan-fu-base F N-oxide (**7**) [11], atisine chloride (**8**) [12], isoatisine (**9**) [12], coryphine (**10**) [13], and coryphidine (**11**) [14].



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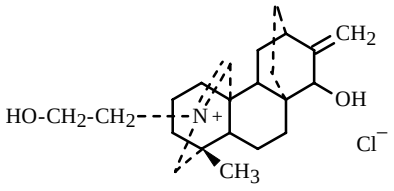

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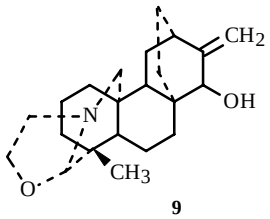

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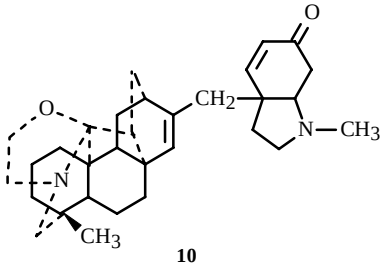
**1:** R = $R_1$ = $R_2$ = H
**2:** R = $COCH_3$, $R_1$ = $R_2$ = H
**3:** R = $COCH_2CH_3$, $R_1$ = $R_2$ = H
**4:** R = $COCH(CH_3)_2$, $R_1$ = $R_2$ = H
**5:** R = $COCH(CH_3)_2$, $R_1$ = $R_2$ = H, N→O
**6:** R = $COCH(CH_3)_2$, $R_1$ = H, $R_2$ = $COCH_3$
**7:** R = $COCH(CH_3)_2$, $R_1$ = H, $R_2$ = $COCH_3$, N→O



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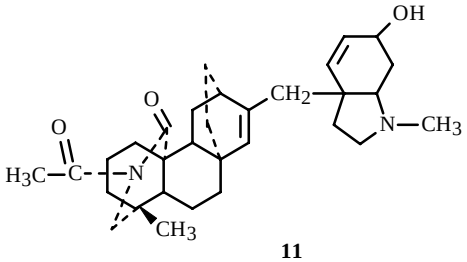

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TABLE 1. Acute Toxicity of Diterpenoid Alkaloids **1**-**12**

| Alkaloid | $LD_{50}$, mg/kg, P = 0.05 |
|---|---|
| Tangutisine (**1**) | 110 (91.7-132 |
| Acorine (**2**) | 48 (43.6-52.8) |
| Acoridine (**3**) | 73 (65.7-81.0) |
| Guan-fu-base Z (**4**) | 57 (50.9-63.2) |
| Guan-fu-base Z N-oxide (**5**) | 230 (205.3-257.6) |
| Guan-fu-base F (**6**) | 50 (43.8-57) |
| Guan-fu-base F N-oxide (**7**) | 225 (187.5-270) |
| Atisine chloride (**8**) | 9 (8.2-9.9) |
| Isoatisine (**9**) | 8 (7.14-9.0) |
| Coryphine (**10**) | 8.1 (7.3-8.9) |
| Coryphidine (**11**) | 20 (17.9-22.4) |
| Methyllycaconitine (**12**) | 3.9 (3.54-4.29) |

The isolated alkaloids belong to the hetisine (**1**-**7**), atisine (**8** and **9**), and coryphine (**10**) types. Coryphine has the atisine skeleton with an additional bridge between C-14 and C-20 and a hexahydro-N-methylindol-6-one fragment on C-17. Coryphidine (**11**) can be viewed as secocoryphine.

We found previously that DA exhibit various toxicities and spectra of pharmacological properties as a function of the heterocyclic skeleton, nature and site of substituents, and stereoelectronic properties of the N atom [15-17].

Such data for pure alkaloids from *A. coreanum* have not been reported.

Here we report results from research on the biological activity of alkaloids isolated from *A. coreanum* and the structure—activity relationship.

Intravenous administration of DA to animals led to similar resorptive action presenting as progressively increasing weakening of skeletal musculature and breathing difficulties. The action of **1**-**11** differed in rate of onset, intensity, manifestation, and duration of resorptive effect. Table 1 contains data for the acute toxicity of these alkaloids upon i.v. administration to white mice.

The toxicity of the alkaloids varies from 8-9 to 225-230 mg/kg. Atisine chloride (**8**), isoatisine (**9**), and coryphine (**10**) are most toxic. Hetisine-type alkaloids (**1**-**7**) are less toxic than **8**-**10** whereas **2**-**4** and **6** with esters are more toxic than aminoalcohol **1**. The least toxic compounds are N-oxide acyl derivatives of 14-hydroxyhetisine (**5** and **7**). This is probably due to the decreased electron density on the N atom.

Judging from the nature of resorptive action and the degree of toxicity, atisine chloride, isoatisine, and coryphine exhibit the highest myorelaxant activity among the *A. coreanum* alkaloids. The curare-like activity and mechanism of action of these alkaloids was investigated using in vitro and in vivo experiments to compare them with mellyctine (methyllycaconitine), which is used in medical practice [18] and is a lycoctonine-type norditerpenoid alkaloid [19].

The most active *A. coreanum* alkaloids (**8**-**10**) are less toxic than mellyctine (**12**) whereas their curare-like activities are similar and in some instances greater (Tables 1 and 2). In contrast with mellyctine, the activity of **8**-**10** in in vitro and in vivo experiments develops and abates more quickly. They have more distinct H-choline blocking and myotropic spasmolytic activity on isolated organs.

The most active alkaloid from *A. coreanum* in various tests was coryphine (**10**) (Table 2). The rate of onset and intensity of muscle-weakening action of **10** in experiments with rabbits was faster than mellyctine. However, the duration of action was shorter. The effective dose causing head tilting was 1.8 mg/kg for coryphine and 2.5 for mellyctine. The acute toxicity upon i.v. administration to mice of **10** was twice as great as that of **12**. The $LD_{50}$ for **10** was 8.1 mg/kg; for **12**, 3.9.

The ability to block the amplitude of muscle contractions of a phrenico-diaphragmal preparation of rat and to suppress acetylcholine contracture of smooth muscle of frog stomach was ten times greater for **10** than **12**. Coryphine at concentrations of $10^{-6}$-$10^{-5}$ g/mL, in contrast with mellyctine, exhibits myotropic spasmolytic activity.

TABLE 2. Comparative Myorelaxant Activity of **8-10** and **12**

| Compound | Dose, mg/kg* | Effective concentrations, g/mL** | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| **8** | 3.5 | - | $7 \cdot 10^{-6}$ | - | $1 \cdot 10^{-4}$ |
| **9** | 2.0 | $5 \cdot 10^{-6}$ | $1 \cdot 10^{6}$ | $5 \cdot 10^{7}$ | $8 \cdot 10^{5}$ |
| **10** | 1.8 | $1 \cdot 10^{-6}$ | $5 \cdot 10^{-7}$ | $1 \cdot 10^{7}$ | $5 \cdot 10^{6}$ |
| **12** | 2.5 | $2 \cdot 10^{-5}$ | $2 \cdot 10^{5}$ | $1 \cdot 10^{6}$ | $> 1 \cdot 10^{4}$ |

*Doses causing head tilting in rabbits.

**Concentrations causing suppression of: neuromuscular conductivity blockade of phrenico-diaphragmal rat preparation (1), acetylcholine contracture of smooth muscle of frog stomach (2), amplitudes of elicited and miniature potentials of frog end plates (3), contracture of rat intestine caused by $BaCl_2$ ($2 \cdot 10^{-4}$) (4).

Intracellular depolarization of membrane potentials in isolated frog skeletal muscle has shown that **10**, like **12**, has no effect on the membrane potential of muscle fiber and frequency of spontaneous miniature potentials but does lower the amplitude of elicited and miniature potentials of end plates, leading to complete blockage of acetylcholine receptors of postsynaptic membrane. The strength and rate of onset of neuromuscular blockage is ten times greater for coryphine than for mellyctine.

Coryphine, like mellyctine, in acute experiments on anesthetized cats and dogs had no noticeable effect on adreno-, serotonine, and histamine receptors, on the frequency of cardiac contractions, and the functioning of cardiovascular systems. It slightly lowered the tone of peripheral vessels, sedated sympathetic and parasympathetic ganglia, elicited brief hypotension, and exhibited a central H-cholinolytic activity in large doses.

Thus, the investigated alkaloids exhibit myorelaxant activity that is more evident for isoatisine and coryphine, which contain oxazolidine rings, and atisine chloride (**8**). Forming the chloride opens the oxazolidine ring of **8**. Hetisane alkaloids **1-7** have lower curare-like activity that decreases in the order acyl derivatives of 14-hydroxyhetisine (**2-4**, **6**) > 14-hydroxyhetisine (**1**) > acyl 14-hydroxyhetisine N-oxide derivatives (**5**, **7**), i.e., the curare-like activity depends on the type of heterocyclic skeleton, the presence of esters, and the electronic properties of the N atom.

Coryphine, which possesses the highest activity in various categories compared with other DA, has a unique heterocyclic structure and the unusual hexahydro-N-methylindol-6-one substituent on C-17. This is probably the reason for its excellent properties.

The experimental data indicate that the toxicity of *A. coreanum* is due mainly to alkaloids that have myorelaxant activity.

Compounds with distinct curare-like activity were previously found among DA containing the lycoctonine skeleton. The myorelaxant activity for hetisine, atisine, and coryphine DA is found for the first time.

## EXPERIMENTAL

The method for preparing **1-11** has been described [1, 7-14]. The pharmacological properties of the alkaloids were investigated in experiments on mice, rats, rabbits, cats, and dogs. The resorptive activity and acute toxicity was studied using generic white mice of mass 18-22 g. The effect of the preparations on systemic arterial pressure, breathing, peripheral part of the nervous system, and EKG was studied in acute experiments on anesthetized (sodium pentobarbital 40 mg/kg, i.p.) cats and dogs as before [20, 21]. The curare-like activity was determined in in vitro experiments on neuromuscular preparations of frog (*Rana temporaria*) sartorius and ishiadicus by intracellular depolarization of potentials, on isolated phrenico-diaphragmal preparation of rat, and in experiments in vivo on conscious rabbits using head tilting. The spasmolytic activity was studied in isolated sections of rat and rabbit intestine treated with $BaCl_2$ ($2 \cdot 10^{-4}$ g/mL).

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