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ISOLATION OF LAPPACONITINE FROM *Aconitum septentrionale* ROOTS BY ADSORPTION

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Extracts of Aconitum septentrionale *Koelle roots obtained using chloroform, isopropanol, and ethanol were purified using chloroform and basic γ-Al_2O_3. Ballast materials were selectively adsorbed by γ-Al_2O_3, increasing the mass fraction of lappaconitine in the extract. The ethanol extract was purified most. The degree of lappaconitine extraction by chloroform was unaffected by the presence of γ-Al_2O_3. However, the mass fraction in the extract and lappaconitine extraction from* Aconitum septentrionale *were increased more than twice.*

Key words: lappaconitine, *Aconitum septentrionale*, diterpene alkaloids, adsorptive isolation, extraction, Al_2O_3.

Aconitum septentrionale Koelle roots are a source of lappaconitine, the hydrobromide of which is a pharmacopeic preparation for antiarrhythmia [1, 2].

At present various separations of this alkaloid from the plant raw materials have been developed. However, the basic principles have not changed. Plant raw material is suitably extracted with benzene [3], chloroform [4], methanol [5], acetone:water [6], ethanol:water [7], or diluted aqueous acids [8]. The resulting extract contains the total alkaloids and a complex mixture of ballast materials. Isolation and purification of lappaconitine is a multi-step extraction procedure that leads to losses of the product.

Use of a mechanical chemical method that consists of preliminary vigorous mechanical processing of plant raw material together with an added solid [9, 10] (oxide adsorbents, mineral salts, organic acids, sugar, urea) increased the yield, rate, and selectivity of the extraction of the product.

In the present work, the role of γ-Al_2O_3 on the degree and selectivity of lappaconitine extraction from *A. septentrionale* roots by $CHCl_3$ was studied in greater detail.

Raw material was extracted with $CHCl_3$, $CHCl_3$ with γ-Al_2O_3, isopropanol, and ethanol. Table 1 lists the analytical results from UV spectroscopy of the resulting extracts.

Quantitative analysis of the ethanol extract showed that the lappaconitine content per mass of air-dried raw material was less than 3.6% according to HPLC and 3.8% according to UV spectroscopy.

Comparison of the analytical data of extracts obtained using $CHCl_3$ found that the presence of γ-Al_2O_3 in the extraction system with $CHCl_3$ had no effect on the degree of lappaconitine extraction. However, it decreased the amount of ballast materials in the extract owing to their adsorption on γ-Al_2O_3. In this instance the adsorbent could not be regenerated because of the complexity of separating it from the processed raw material.

The yield of extractive substances increased sharply if the solvent polarity was increased in the order $CHCl_3$-*i*-PrOH-EtOH. The amount of extracted lappaconitine also increased. However, the selectivity of the extraction decreased. This indicated that most of the ballast materials were polar.

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TABLE 1. Extract Yields and Amounts of Lappaconitine in Them After Single Extraction of Raw Material by Various Solvents

Expt. No.	Solvent, sorbent	Extract yield, mass % of raw matl.	Sample No.	Amount of lappaconitine in extract, mass % of extract (mass % of raw matl.)
1	$CHCl_3$	5.4	I	48 (2.6)
2	$CHCl_3$, γ-Al_2O_3	4.1	III	63 (2.6)
3	*i*PrOH	7.8	IV	39 (3.0)
4	EtOH	19	VI	20 (3.8)

TABLE 2. Extract Yields and Amounts of Lappaconitine in Them After Adsorption Purification

Expt. No.	Yield, mass % of raw matl.	Sample No.	Amount of lappaconitine, mass % of extract (mass % of raw matl.)
1	3.5	II	69 (2.4)
3	3.7	V	77 (2.8)
4	4.2	VII	84 (3.5)

The extracts could be effectively purified using basic γ-Al_2O_3 and a weakly polar solvent. Because of the presence of basic centers on the surface of γ-Al_2O_3 and the qualitative features of nonelectrolyte adsorption on polar adsorbents [11], it was expected that γ-Al_2O_3 would adsorb from weakly polar solvents primarily the most polar substances that tend to form H-bonds and possess acidic properties, like the majority of the ballast materials. Alkaloids, if they are weakly polar bases, would remain in solution. Thus, the degree of adsorption of polar ballast materials should increase if the solvent polarity decreases. Therefore, we used $CHCl_3$ for the adsorption purification.

Dry extracts obtained using $CHCl_3$ (without γ-Al_2O_3), isopropanol, and ethanol were dissolved in $CHCl_3$ and adsorbed onto γ-Al_2O_3. Part of the material from the alcohol extracts did not dissolve in $CHCl_3$. Table 2 lists the analytical data of the mixtures obtained after separating the solids and removing solvent. According to Table 2, ballast materials were selectively removed from the extracts by the procedure. Adsorptive purification of the ethanol extract was the most selective and gave the highest product yield. The high (84%) lappaconitine content in the mixture enabled it to be crystallized without further concentration (yield after a single crystallization was 3.6% of the raw material mass).

The solid that separated during adsorptive purification of the ethanol extract and consisted of extracted materials that were insoluble in $CHCl_3$ and γ-Al_2O_3 with ballast materials adsorbed on it, was treated with EtOH, resulting in dissolution and desorption. The resulting solution was tested qualitatively for alkaloids, giving a negative reaction. This indicates that they were completely separated. Thus, the sorbent can be regenerated, giving ballast materials that can possibly be used as a complex of biologically active substances.

The described method for purification of the extracts can be used to isolate lappaconitine from *A. septentrionale* roots. When compared with extractive purification, it has fewer steps, uses less solvent per unit mass of product, and does not use acids and bases. Apparently the method is especially effective if the lappaconitine content in the raw material is high.

EXPERIMENTAL

Plant material (air-dried *A. septentrionale* K. roots collected in Altai Territory) was finely ground beforehand in a Fritsch Pulverizette planetary mill (2 min, setting 10). Chemically pure solvents were used. The sorbent was basic γ-Al_2O_3 for column chromatography. The specific surface area 150.9 m^2/g was determined by N_2 absorption; the number of basic centers (Hammett strength > 9.2) 275 ± 10 μmol/g; by titration of a $CHCl_3$ suspension of γ-Al_2O_3 with benzoic acid using phenolphthalein indicator. Spectrophotometric measurements were made on a Shimadzu UV-240 spectrometer; quantitative determination of lappaconitine, by HPLC on a Milichrom 4 instrument with a UV detector, Nukelosil C-18 column (5 μm), and elution using AcCN:Tris (0.1 M) (4:1 and 1:1, 700 μL each) at flow rate 100 μL/min. The melting point of lappaconitine was determined on a Kofler block.

1. Extraction of Raw Material by $CHCl_3$. Adsorptive Purification of the Extract. Raw material (25.42 g) was extracted once with $CHCl_3$ (300 mL) with stirring for 8 h at 45-50°C and 5 h at 22-24°C. Filtration and removal of solvent in vacuo produced a dry glassy extract (1.36 g). A sample (I, 0.06 g) was analyzed for lappaconitine content. The remaining extract was dissolved completely in $CHCl_3$ (300 mL), treated with γ-Al_2O_3 (7.29 g), and adsorbed with stirring for 8 h at 45-50°C and 5 h at 22-24°C. Separation of sorbent by filtration and removal of solvent in vacuo produced a dry solid (II, 0.84 g).

2. Extraction of Raw Material by $CHCl_3$ with γ-Al_2O_3. A mixture of raw material (25.39 g) and γ-Al_2O_3 (7.62 g) was extracted once with $CHCl_3$ (300 mL) with stirring for 8 h at 45-50°C and 5 h at 22-24°C. Filtration and removal of solvent in vacuo produced a dry glassy extract (III, 1.04 g).

3. Extraction of Raw material by Isopropanol. Adsorptive Purification of the Extract. Raw material (29.31 g) was extracted once with *i*-PrOH (350 mL) with stirring at 45-50°C for 9 h. Filtration and removal of solvent in vacuo produced a dry extract (2.28 g). A sample (IV, 0.11 g) was analyzed for lappaconitine content. The remaining extract was treated with $CHCl_3$ (150 mL, incomplete dissolution) and γ-Al_2O_3 (3.48 g) and adsorbed with stirring at 22-24°C for 1.5 h. Filtration and removal of solvent in vacuo produced a dry solid (V, 1.03 g).

4. Extraction of Raw Material with Ethanol. Adsorptive Purification of the Extract. Isolation of Lappaconitine. Desorption. Raw material (25.05 g) was defatted with petroleum ether (100 mL) for 30 min. The mass of removed resins was 0.23 g. It was extracted once with ethanol (300 mL) with sitrring at 45-50°C for 9 h. Filtration and removal of solvent in vacuo produced a dry powdery extract (4.80 g). A sample (VI, 0.11 g) was analyzed for lappaconitine content. The remaining extract was treated with $CHCl_3$ (300 mL, incomplete dissolution) and γ-Al_2O_3 (7.5 g) and adsorbed for 1.5 h with stirring at 22-24°C. Removal of the solid by filtration and removal of solvent in vacuo produced a dry solid (1.03 g). A sample (VII, 0.02 g) was analyzed for lappaconitine content. Addition to the dry solid of acetone (2 mL) produced a precipitate of lappaconitine (0.89 g). A series of recrystallizations from acetone and acetone:$CHCl_3$ (1:1) afforded crystalline lappaconitine (A, 0.45 g), mp 219°C. The solid that separated earlier during adsorptive purification and consisted of γ-Al_2O_3, adsorbed ballast materials, and compounds insoluble in $CHCl_3$, was washed with $CHCl_3$ (100 mL, 0.008 g of removed compounds) and dissolved and desorbed twice with stirring by portions of EtOH (100 mL each) at 45-50°C. The filtrates were combined. Solvent was removed in vacuo to afford a dry solid (B, 3.21 g) that did not contain alkaloids (negative reaction with silicotungstic acid).

Spectrophotometric Determination of Lappaconitine Content in Extracts and Intermediate Mixtures (I-VII). UV absorption spectra of samples $\varepsilon(\lambda)$ ($L \cdot g^{-1} \cdot cm^{-1}$) were recorded using linear combinations $\varepsilon_c(\lambda)$ of lappaconitine spectra [A, $\varepsilon_A(\lambda)$] and mixtures of ballast materials [B, $\varepsilon_B(\lambda)$] (EtOH, c = 0.05-0.03 g/L, λ = 200-360 nm): $\varepsilon_C(\lambda) = X \cdot \varepsilon_A(\lambda) + Y \cdot \varepsilon_B(\lambda)$, where X and Y were determined by least-squares methods. The quantity $X \cdot 100\%$ is the mass fraction of lappaconitine in the sample. In all instances the $\varepsilon(\lambda)$ and $\varepsilon_C(\lambda)$ curves agreed with satisfactory accuracy. The relative experimental uncertainty was 7%.

Determination of Lappaconitine Content in Alcohol Extract (VI) by HPLC. Three solutions of lappaconitine (A) of concentrations 1.032, 0.688, and 0.344 mg/mL were used for calibration. Chromatograms were recorded at 250 nm. The calibration curve was $C = (0.0089 \pm 0.0001) \cdot S$, where C is the lappaconitine concentration in mg/mL and S is the peak area. The retention time of lappaconitine was 9.93 ± 0.03 min. The chromatogram of the solution of extract (VI) (c = 1.933 mg/mL) gave a peak for lappaconitine with a retention time of 9.99 min. The calculated lappaconitine concentration was 0.367 ± 0.005 mg/mL; the content in the extract, 19 ± 0.3 mass%.

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