METHOD FOR THE QUANTITATIVE DETERMINATION OF ADLUMINE IN *Corydalis sempervirens* AND AS THE ISOLATED SUBSTANCE

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A chromato-spectrometric method has been developed for the quantitative determination of the biologically active alkaloid adlumine of pale corydalis which permits the determination of adlumine in the plant material with an accuracy of ±3.62% and of the isolated substance with an accuracy of ±0.79%.

Adlumine is a phthalide-isoquinoline alkaloid with the composition $C_{21}H_{21}NO_6$, mp 179-180°C, $[\alpha]_D^{21} - 42°$ (c 1.2; chloroform) the structure of which was established by Manske. It has been detected in many species of *Corydalis* [1-5]. The raw material for the isolation of adlumine is *Corydalis sempervirens* Pers. (pale corydalis), family Papaveraceae. In addition to adlumine, the total alkaloids of pale corydalis contain protopine, cryptopine, adlumidine, bicuculline, adlumiceine, and hydroxysanguinarine.

According to the pharmacology and folk medicine laboratory of VILR [All-Union Institute of Medicinal Plants], adlumine sulfate is a biologically active substance. The material is undergoing pharmacological investigations.

To monitor the quality of the corydalis raw material and to determine adlumine sulfate as such, the necessity has arisen for the development of quantitative estimation.

In the development of a method of analysis in the raw material, the extraction of the combined alkaloids, their chromatographic separation in a thin layer of sorbent, the elution of the free adlumine from the sorbent, and its subsequent spectrophotometry have been studied.

As the solvents for the extraction of the alkaloids from the raw material we have tested chloroform, benzene, and dichlorethane with the addition of ammonia, sulfuric acid, ethanol, and toluene with the previous wetting of the raw material with water. Extraction was carried out by various methods: in a Soxhlet apparatus, by boiling in a flask under reflux on the water bath, and by steeping at room temperature. As a result, we adopted the method of steeping the raw material with toluene at room temperature.

To purify the combined alkaloids, extraction of the toluene solutions with 5% sulfuric acid followed by their reextraction in benzene has been proposed.

The main task in separating the combined alkaloids with the aid of chromatography in a thin layer of sorbent amounts to the separation of the alkaloids adlumine and adlumidine, which are close in their properties. Various systems of solvents and sorbents were tested. The best separation of adlumine and adlumidine and other accompanying substances was achieved on type KSK 20 silica gel as a fixed alkaline layer in the ether–chloroform (4:1 system). When the silica gel plate was observed in UV light, zones of adlumine with $R_f \sim 0.63$, of adlumidine with $R_f \sim 0.71$, and of bicuculline with $R_f \sim 0.39$ appeared. The individuality of the adlumine zone followed from the identity of the UV, IR, and NMR spectra of eluates of the zones obtained in the chromatography of pure adlumine and in the chromatographic separation of the combined alkaloids.

The UV spectrum of free adlumine (the solvent being ethanol) has adsorption maxima at 290 and 326 nm. The maximum at 326 nm is the most convenient for performing quantitative determination.

The metrological characteristics of the method were established from the results of a determination of free adlumine in the raw material with ten repetitions:

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f	$\bar{x}$	S^2	S	P	$t(p,f)$	Δx	$\pm\varepsilon$	$\pm\bar{\varepsilon}_3$
9	0.251	0.48×10^{-4}	0.696×10^{-2}	95	2.26	0.016	6.267	3.62

The relative accuracy of the method with three determinations is ±3.62%.

To reveal any systematic error of the method, experiments were performed with additions of pure free adlumine to the plant raw material. The analyses were performed in three independent repetitions:

Amount of free adlumine in 4 g of raw material, mg	Free adlumine added, mg	Calculated, mg	Found, mg	Relative error, %
18,25	—	18,25	18 25	0
18,25	1,86	20 11	19 96	—0,75
18,25	2,53	20,78	20,09	—3,32
18,25	3,64	21,89	21,12	—1,05

As we see, the relative error of the determination of adlumine in the plant raw material when the analysis is performed in triplicate is within the limits of the random error, and therefore the procedure is recommended to include analysis in triplicate.

The basis of the method for the quantitative determination of adluminine sulfate as such has been made the spectrophotometric determination of the optical density of an aqueous solution of it. The UV spectrum of adlumine sulfate in water has an absorption maxima at a wavelength of 328 nm.

To check the reproducibility of the method, a quantitative determination of one sample of the substance was made in quintuplicate. The following metrological characteristics of the method were obtained:

f	x	S^2	S	p	(p, f)	Δx	ε	ε_3
4	99,59	0,16	0,4	95	2,78	1,11	1,12	±0,79

Consequently, the relative error of the method of determining adlumine sulfate as such in duplicate amounts to ±0.79%.

EXPERIMENTAL

Determination of Free Adlumine in the Raw Material. An analytical sample (5 g of ground plant with a particle size of 1-2 mm) was wetted with 6 ml of water and was left to swell for 30 min, after which 250 ml of toluene was added, the mixture was carefully stirred, and steeping was carried out for 15-20 h. The toluene extract was filtered through a paper filter, 200 ml of the extract was taken, and the alkaloids were reextracted with a 5% solution of sulfuric acid (4 × 50 ml). The combined sulfuric acid extracts were made alkaline with 25% ammonia to pH 8-9 (universal indicator) and the alkaloids were reextracted with benzene (3 × 100 ml). The benzene extract was dried with sodium sulfate and evaporated to dryness. The dry residue was dissolved in 5 ml of benzene.

Three bands of 0.04 ml of the benzene extract each were deposited with a micropipette (the length of each line was 2 cm) at the starting line of a chromatographic plate (20 × 20 cm), together with three bands of the same length each containing 0.02 ml of a solution of a standard sample of adlumine, and a band was left for a control experiment. The plate was dried in the air for 10 min. Chromatography was performed at room temperature in a vertical chamber that had first been saturated for 20 min. The mobile phase was ether—chloroform (4:1). After the solvent front had migrated for 19 cm, the plate was taken from the chamber and dried in the air for 30 min; it was then viewed in UV light at a wavelength of 360 nm, the zones of adsorption of free adlumine were marked, and an amount of sorbent equal to the area of the zone was taken from the control band. The silica gel was transferred from the marked sections quantitatively into a 25-ml flask with a ground-in stopper, 10 ml of 95% ethanol was added, and the free adlumine was eluted with shaking on a shaking machine for 2 h. The eluates were filtered through blue-band ash-free filters. The optical densities of the eluates were measured on a spectrophotometer at a wavelength of 326 nm in a cell with a layer thickness of 10 mm. The eluate from the control band was used as the comparison solution.

By making use of an average value of the optical density of the eluates of a solution of the standard sample the total amount of free adlumine in each of the solutions investigated was calculated as a percentage of the absolutely dry mass.

Determination of Adlumine Sulfate as Such. An analytical sample of about 0.0200 g (accurately weighed) of the material was dissolved in a 500 ml measuring flask in 300 ml of water that had been heated to 30-35°C. After the solution had cooled to room temperature, its volume was made up to the mark with water and it was carefully mixed. The optical density of the solution obtained was measured on a spectrophotometer at a wavelength of 328 nm in a cell with a layer thickness of 10 mm. Water was used as the comparison solution.

In parallel, the optical density of a solution of a standard sample of adlumine sulfate was measured under the same conditions.

By using the average value of the optical density of the solution of the standard sample the percentage purity of the adlumine sulfate was calculated.

CONCLUSION

A spectrophotometric method for determining adlumine as such with an accuracy of ±0.79% and a chromato-spectrophotometric method for determining free adlumine in plant raw material with an accuracy of ±3.62% has been developed.

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THE CHANGE IN THE GLOBULINS DURING THE DEVELOPMENT OF COTTON SEEDS

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The biosynthesis of the 11S and 7S globulins — the main reserve proteins of cotton seeds — has been investigated. The periods at which the globulins appear in cotton seeds have been established. The changes in the amino acid composition and in the secondary structure of the 11S globulin during the ripening of cotton seeds have been studied.

The globulins of cotton seeds consist of two components — 11S (histidine) and 7S (arginine) proteins. The quantitatively predominating 11S globulin makes up ∿70% of the total globulin fraction [1]. We are performing investigations of the 11S globulin with the following aims:

1) to elucidate features of the quaternary structure of the protein and its link with the primary structures of the individual subunits in order to establish the roles of these structures in the appearance of the basic function of the reserve protein; and

2) to study the properties of the 11S globulin and their interrelationships with its structure for regulating the functional properties necessary in the production of edible cottonseed protein.

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