# SPECTROPHOTOMETRIC DETERMINATION OF THE CARDENOLIDES IN THE EPIGEAL PART OF Adonis chrysocyathus

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It is known that Adonis chrysocyathus Hook. et f. Thom contains valuable cardenolides – k-strophanthin-$\beta$ and cymarin [1]. Recently a new tetraoside – sugoroside – has been detected in this plant [2].

The present paper gives a method for analyzing the epigeal part of A. chrysocyathus collected in the environs of the village of Shakhimardan in the Khurdan district of the Alai range. The essence of the method consists in the extraction of the cardenolides from the raw material with 96% ethanol, the chromatographic separation of the glycosides to be determined in a nonfixed layer of alumina, and their spectrophotometric determination [3].

By comparing various methods of extraction (Table 1) it was shown that it is more effective timewise to extract the glycosides by boiling the raw material with 96% ethanol. A smaller amount of ballast substances is extracted by this method.

Since the epigeal part of the plant contains a large amount of ballast substance (pigments, chlorophyll, resins), which complicates analysis, and their removal by known methods [4] leads to a loss of glycosides, we used double chromatography. The first plate with the deposited extract was chromatographed in diethyl ether, which purified the extract. The second chromatographic process in the water-saturated

TABLE 1. Amount of Cardenolide (% on the absolutely dry weight of the raw material)

| Method of extraction | 70% ethanol | | | 96% ethanol | | |
|---|---|---|---|---|---|---|
| | k-strophanthin-$\beta$ | cymarin | total | k-strophanthin-$\beta$ | cymarin | total |
| | | | 1970 crop | | | |
| Hot extraction for 2.5 h | 0,119 | 0,521 | 0,640 | 0,119 | 0,514 | 0,633 |
| | 0,120 | 0,520 | 0,640 | 0,118 | 0,519 | 0,637 |
| Cold extraction for 23 h | 0,060 | 0,421 | 0,481 | 0,053 | 0,402 | 0,455 |
| | 0,061 | 0,412 | 0,473 | 0,054 | 0,403 | 0,457 |
| | 0,064 | 0,413 | 0,477 | 0,058 | 0,401 | 0,459 |
| | — | — | — | 0,055 | 0,400 | 0,455 |
| In a Soxhlet apparatus for 24 h | — | — | — | 0,117 | 0,518 | 0,635 |
| | — | — | — | 0,117 | 0,521 | 0,638 |
| | | | 1971 crop | | | |
| Hot extraction for 2.5 h | 0,175 | 0,196 | 0,371 | 0,175 | 0,193 | 0,368 |
| | 0,178 | 0,205 | 0,383 | 0,176 | 0,195 | 0,371 |
| | — | — | — | 0,175 | 0,193 | 0,368 |
| | — | — | — | 0,175 | 0,197 | 0,372 |
| Cold extraction for 23 h | 0,131 | 0,164 | 0,295 | 0,127 | 0,119 | 0,246 |
| | 0,134 | 0,161 | 0,295 | 0,127 | 0,120 | 0,247 |
| | 0,132 | 0,161 | 0,293 | 0,132 | 0,119 | 0,251 |
| | 0,130 | 0,167 | 0,297 | 0,130 | 0,111 | 0,241 |

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TABLE 2

| No. of the expt. | Content of k-strophanthin-β in the extract | Added standard k-strophanthin-β | Calculated | Found | Absolute error | Relative error |
|---|---|---|---|---|---|---|
| | mg | | | | % | |
| 1 | 0,438 | 0,254 | 0,692 | 0,668 | −0,024 | 3,6 |
| 2 | 1,614 | 0,136 | 1,750 | 1,728 | −0,021 | 1,20 |
| 3 | 1,614 | 0,135 | 1,749 | 1,729 | −0,020 | 1,14 |
| 4 | 0,720 | 0,192 | 0,912 | 0,890 | −0,022 | 2,41 |
| 5 | 0,580 | 0,148 | 0,728 | 0,740 | +0,012 | 1,65 |

butanol (1 : 1) system ensured the separation of the cardenolides. The cardenolides were eluted in the usual way: the k-strophanthin-β with 70% ethanol and the cymarin with 96% ethanol.

As the complex-forming reagent for the quantitative determination of the cardenolides in place of the known reagent 2,4-dinitro diphenyl sulfone [3] we used 2,2',4,4'-tetranitrobiphenyl [5, 6]; with cardenolides the latter forms a stable complex (time of constancy of the maximum 10-15 min) which increases the accuracy of the determination. The optical densities of the eluates were measured on an SF-4 instrument at $\lambda_{max}$ 610 nm. The accuracy of the method was checked by analyzing extracts with a standard solution of k-strophanthin-β added to them (Table 2).

## EXPERIMENTAL

To a weighed sample of about 20 g (*a*) of the comminuted epigeal part of A. chrysocyathus was added 100 ml of 96% ethanol, and the mixture was boiled on the water bath for 30 min. The extract was carefully poured through a filter into another flask and the extraction was repeated twice more, increasing the time of boiling to 60 min. The combined extract was evaporated in vacuum at 40-50°C to a volume of about 10 ml. The concentrate was carefully transferred by means of a pipette into a measuring cylinder, the flask was washed several times with 5-ml portions of hot methanol, and, after cooling, the final volume was measured ($V_{ex}$). A plate with a nonfixed layer of alumina was divided into two parts. On each band 1.0-1.5 ml (b) of the extract was deposited and, after drying, the plate was placed at an angle of 20-30° in a chamber with diethyl ether. After the solvent front had traveled 20 cm, the plate was removed from the chamber and the ether was allowed to evaporate off in the air. The dried plate was placed in another chamber and chromatographed in the water-saturated butan-1-ol (1 : 1) system. The distance of migration of the solvent front was 17 cm. After the solvent had evaporated off from the plate, one half of it was treated with Raymond's reagent and the zones corresponded to the k-strophanthin-β and cymarin on the untreated band of the plate were marked out. The portions of alumina with the adsorbed cardenolides were transferred to columns (h 20, d 2 cm) and eluted. The volume of eluate ($V_{el}$) depends on the amount of raw material and is determined experimentally.

To determine the amount of cardenolide, to 2 ml of eluate was added 2.5 ml of a 0.15% ethanolic solution of tetranitrobiphenyl and 0.5 ml of a 0.1 N aqueous solution of caustic potash. Simultaneously, in another flask a control solution (blank test) was prepared by mixing 2 ml of 70% or 96% ethanol (eluate from the same batch of alumina but without cardenolide), 2.5 ml of a 0.15% ethanolic solution of tetranitrobiphenyl, and 0.5 ml of 0.1 N aqueous caustic potash. After the addition of the alkali, the solutions were poured into quartz cells (1 cm thick) and immediately placed in the chamber of the spectrophotometer and the maximum value of the optical density, which generally appeared after 15-20 min, was determined. A mixture of standard solutions of k-strophanthin-β and cymarin was also chromatographed and the optical densities of their eluates from the alumina were measured. The percentage of cardenolide (x) in the epigeal part of A. chrysocyathus referred to the absolutely dry raw material was calculated from the formula

$$x = \frac{D_t \, C_{st} \, V_{el} V_{ex} \cdot 10000}{D_{st} \cdot a \cdot b \, (100 - h)},$$

where $D_t$ and $D_{st}$ are the optical densities of the test and standard solutions of the cardenolide; $C_{st}$ is the weight of the standard sample, g; b is the volume of concentrated extract of plant raw material deposited on the chromatogram, ml; *a* is the weight of the raw material, g; $V_{ex}$ is tht total volume of extract, ml; $V_{el}$ is the volume of eluate (test solution), ml; and h is the moisture content of the plant raw material, %.

## SUMMARY

A spectrophotometric method for determining cymarin and k-strophanthin-$\beta$ in the epigeal part of Adonis chrysocyathus has been developed.

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