3-EPISTROPHANTHIDIN AND 3-EPISTROPHANTHIDOL

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As is well known [1–7] the seeds of Strophanthus kombé Oliv. contain a large variety of cardiac glycosides the aglycone fraction of which consists mainly of strophanthidin. There are also glycosides of strophanthidol, periplogenin, and 17β-H-strophanthidin. Obtaining from the glycosides of this plant the strophanthidin which we required for synthetic work, on chromatographing the mother solutions we isolated not only an additional amount of this aglycone but also strophanthidol, 17β-H-strophanthidin, and two other, more polar, cardenolides. The latter were provisionally denoted by the symbols IM-38 and IM-40. Judging from the yields, the plant contains very small amounts of them: from 7 kg of seeds we obtained 110 mg of IM-38 and 15 mg of IM-40. The presence of periplogenin was established only by paper chromatography—it was eluted from a column together with anhydrostrophanthidin.

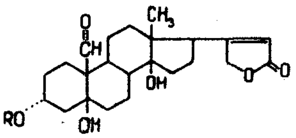
The substances IM-38 and IM-40 give positive reactions for cardenolides and a negative reaction with tetranitromethane. The colors they form with Jensen's reagent characterize a normal β-position of the butenolide rings. The empirical formulas of the substances are, respectively, $C_{23}H_{32}O_6$ and $C_{23}H_{34}O_6$, which permit them to be regarded as aglycones.

The UV spectrum of the aglycone IM-38 has two absorption maxima, at 216 and 303 mμ (log ε 4.18, 1.37). The first of them shows that the substance contains a butenolide ring and the second that it contains an aldehyde group. The presence of an aldehyde group is confirmed by the formation of an acid on oxidation with potassium permanganate. Under the action of acetic anhydride in pyridine, the aglycone forms a monoacetyl derivative with composition $C_{25}H_{34}O_7$. In the region of the stretching vibrations of hydroxyl groups, the IR spectrum of the monoacetate shows an absorption band with a maximum at 3590 cm^{-1}. The molar absorption coefficient of the band is 104.4 $mole^{-1} \cdot l \cdot cm^{-1}$. This corresponds to the absorption of two tertiary (nonacetylating) OH groups.

The optical rotatory dispersion spectrum of the cardenolide IM-38 is characterized by a negative Cotton effect, which shows the cis linkage of the A/B rings [8]. An analysis of the rate of acetylation of the substance by a published method [9] shows that the hydroxyl group that undergoes acetylation is equatorial.

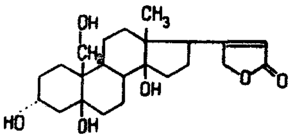
The results obtained permit the assumption that the cardenolide IM-38 is 3-epistrophanthidin (I). The relatively high polarity (see Experimental) of cardenolide IM-38 and its IR spectrum also agree with the proposed structure (I). In the region of the stretching vibrations of OH groups there is an absorption band with a maximum at 3620 cm^{-1} which apparently characterizes free hydroxyl groups. In contrast to the spectrum of strophanthidin obtained under similar conditions, the aglycone IM-38 has no absorption bands at 3480 cm^{-1}, which shows the absence from it of intramolecular hydrogen bonds between the hydroxyls at $C_{(3)}$ and $C_{(5)}$ [10].

In the UV region cardenolide IM-40 shows one absorption maximum, at 216 mμ (log ε 4.19). Oxidation with chromic anhydride forms a cardenolidecarboxylic acid, which shows the presence of a primary alcohol group in the initial substance. The acetylation of the aglycone and an analysis of the rate of the reaction [9] indicates that it contains two acetylatable OH groups—one primary and one secondary and equatorial. Taking these results into consideration and also the elementary composition of the substance and its high polarity, it could be assumed that it is 3-epistrophanthidol (III). This assumption was confirmed by the reduction of 3-epistrophanthidin (IM-38) with sodium borohydride. The reaction, like the preceding one for this compound, was carried out on the microscale. When the reduction product and the cardenolide IM-40 were compared by paper chromatography, they proved to be identical.



I R = H

II R = Ac



III

3-Epistrophanthidin (I) and 3-epistrophanthidol (III) have apparently been obtained for the first time. The presence of 3-epicardenolides in plants is a comparatively rare phenomenon. So far only five such substances have been known: 3-epidigitoxigenin [11, 12], carpogenin, carpogenol [13], 3-epicorotoxigenin [14], and 3-episarmentogenin [15]. The question remains open as to whether plants contain enzymes which convert the "normal" cardenolides into the 3-epi compounds or whether the latter are synthesized in the development of the plants simultaneously with the 3-β-O-aglycones. It is appropriate to mention that the 3-epistrophanthidin (I) and 3-epistrophanthidol (III) were obtained from raw material which had been stored for about 15 years.

EXPERIMENTAL

The substances were analyzed after being dried over phosphorus pentoxide in vacuum (0.01 mm Hg) at 110° C for 2 hr. The paper chromatography of the cardenolides was carried out with the solvent systems: methyl ethyl ketone—m-xylene (1 : 1)/formamide and chloroform—tetrahydrofuran (1 : 1)/formamide. In the first system the cardenolides isolated had the following $R_{\text{strophanthidin}}$ values (strophanthidin 1.00): 17β-H-strophanthidin 0.72; strophanthidol 0.49; 3-epistrophanthidin (IM-38) 0.16; and 3-epistrophanthidol 0.07.

The IR spectra of the substances were obtained by I. P. Kovalev on a UR-10 spectrometer for solutions in methylene chloride, and the optical rotatory spectra on a SPU-E automatic spectropolarimeter. The IR absorption spectra were determined on a EPS-3 spectrometer.

The comminuted strophanthus seeds (7 kg) were defatted with petroleum ether and the powder was dried. The cardenolides were extracted completely with ethanol from the raw material prepared in this way. The extracts were evaporated in vacuum. The still residue was freed from residues of oil and chlorophyll with ethyl ether (1.5 *l* × 4), after which it was dissolved in 8 *l* of ether—chloroform (1 : 2). This solution was treated with water (0.3 *l* × 2). The aqueous layer was separated off and the glycosides which had partially passed into it were extracted with the pure organic solvent (0.2 *l* × 2). The combined ethanolic-chloroformic extracts were evaporated to dryness in vacuum. The residue, consisting of the purified total strophanthus cardenolides, was dissolved in 4 *l* of hot ethanol, and the solution was treated with 4 *l* of 0.4 N aqueous HCl and heated to 80° C. The solution was left at 20–25° C for 7 days, the degree of hydrolysis being checked chromatographically on paper. Then 12 *l* of chloroform was added to the mixture, which contained a crystalline precipitate of aglycone, and it was carefully stirred for 30 min. The ethanolic-chloroformic layer was separated off and the aqueous layer was additionally treated with 5 *l* of chloroform—ethanol (3 : 1). The combined ethanolic-chloroformic extracts were washed with 0.2 *l* of a 1 N solution of sodium carbonate and with water (0.2 *l* × 3) after which they were evaporated in vacuum. The still residue, which was beginning to crystallize, was dissolved with heating in 5 *l* of ethanol—acetone (1 : 2). The solution was concentrated in a hot water bath (without vacuum) to a volume of about 1.3 *l*. The crystals of strophanthidin that deposited under these conditions were separated off and washed with 0.2 *l* of ethanol. The mother solution was concentrated to a volume of about 0.5 *l*. An additional amount of crystalline strophanthidin was obtained, and this was recrystallized from ethanol. In this way 110 g of technical strophanthidin containing a small amount of strophanthidol as impurity was obtained. The mother solutions were evaporated to dryness in vacuum. The residue (50 g) was chromatographed on a column containing 2 kg of alumina (activity grade III).

The cardenolides were extracted with chloroform—ethanol (98 : 2—95 : 5). The fractions containing definite individual substances were evaporated and the cardenolides were crystallized from suitable solvents (see below).

Periplogenin. Obtained in admixture with anhydrostrophanthidin.

17β-H-Strophanthidin. This crystallized from ethanol in the form of thick prisms melting at 225–258/263–268° C; $[\alpha]_D^{24} + 39.2 \pm 3°$ (c 0.50; methanol). Its properties, and also paper chromatography, a mixed melting point, and the color reaction with conc H_2SO_4, showed that the substance was identical with 17β-H-strophanthidin obtained by synthesis.

Strophanthidin. The technical aglycone (120 g) melted at 168–175° C; $[\alpha]_D^{23}$ + 43.2 ± 3° (c 0.54; methanol). It was identified as strophanthidin by paper chromatography, a mixed melting point, and a color reaction with conc H_2SO_4.

Strophanthidol. This crystallized from acetone; it melted at 139–143° C; $[\alpha]_D^{25}$ + 36.2 ± 3° (c 0.74; methanol). A direct comparison of this cardenolide with a sample of strophanthidol showed their identity.

3-Epistrophanthidin (I, IM-38). The substance crystallized from ethanol in the form of coarse prisms melting at 239–257° C; $[\alpha]_D^{25}$ + 42.5 ± 2° (c 1.00; methanol). It dissolved in conc H_2SO_4 forming a coloration changing with time; red (0 min), yellow (2), and lemon-yellow (60 min).

Found, %: C 67.97; H 8.12; mol. wt. 406.2. Calculated for $C_{23}H_{32}O_6$, %: c 68.29; H 7.97; mol. wt. 404.5.

The 3-epistrophanthidin (40 mg) was acetylated with acetic anhydride in pyridine, and chromatography on alumina gave 41 mg of the acetate (II) in the form of a white amorphous powder. 3-Epistrophanthidin acetate has $[\alpha]_D^{24}$ + 42.3 ± 4° (c 1.00; methanol); with conc H_2SO_4 it forms the following colors: yellow (0 min), orange (1 min), red-orange (40 min), brown (180 min), and green (220 min).

Found, %: C 67.03; H 7.80; mol. wt. 443.2. Calculated for $C_{25}H_{34}O_7$, %: C 67.25; H 7.68; mol. wt. 446.5.

To a mixture of 3 mg of 3-epistrophanthidin and 2 mg of sodium borohydride was added 0.2 ml of 80% dioxane, and the solution was left at 20° C for 15 min. After this time, as shown by paper chromatography, the aglycone had been completely reduced. The more polar cardenolide formed had the same "mobility" as substance IM-40.

3-Epistrophanthidol (III, IM-40). The cardenolide crystallized from acetone; it melted at 263–267° C; $[\alpha]_D^{23}$ + 29.2 ± 3° (c 0.62; methanol). With conc H_2SO_4 it formed the following colors: yellow-brown (0 min), red (1 min), red-orange (5 min), pale pink (150 min), and light brown (225 min).

Found, %: C 67.71; H 8.52; mol. wt. 409.3. Calculated for $C_{23}H_{34}O_6$, %: C 67.95; H 8.43; mol. wt. 406.5.

CONCLUSIONS

The hydrolysis of the total glycosides of the seeds of Strophanthus kombé Oliv. and the adsorption-chromatographic separation of the mixture of aglycones has given strophanthidin, strophanthidol, 17β-H-strophanthidin, and two new cardenolides denoted by the symbols IM-38 and IM-40 in the individual state. On the basis of the investigations carried out, the new cardenolides have been characterized provisionally as 3-epistrophanthidin and 3-epistrophanthidol.

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