CARDENOLIDES OF Strophanthus kombe. II*

I. F. Makarevich

UDC 547.918:547.926+615.711.5

Up to the present time, Strophanthus kombe Oliv. has yielded 11 cardiac glycosides: k-strophanthoside [2], k-strophanthin-β [3], cymarin [3, 4], cymarol [4, 5], periplocymarin [4], emicymarin [4, 7], 17βH-emicymarin [4], erysimin (helveticoside) [4, 8], 7βH-helveticoside [4], erysimoside [8], and erysimosol [8]. Leucoerysimoside has also been detected [8], but it has not been obtained in the pure state and its structure, in particular the configuration of the glycosidic linkage and the site of attachment of the terminal D-glucose, has not been established.

In spite of the considerable number of substances isolated from Strophanthus, the full characterization of its cardenolide composition is not yet complete. A chromatographic analysis has shown that the seeds of Strophanthus kombe contain not less than 30 cardenolides. The new compounds that we have recently obtained from this plant [1] – 3-epistrophanthidin and 3-epistrophanthidol – confirm the necessity for further investigations.

The present paper gives the results of the isolation and study of 12 cardiac glycosides. In the choice of procedure, the preparation of native substances was aimed at. The separation into individual components was effected by partition chromatography in columns of silica gel and cellulose. The glycosides obtained have been denoted provisionally by the symbols S 1, S 2, ... S 12. The separation of glycosides having similar polarities, particularly S 9, S 10, and S 11, was very difficult, which made a series of repeat chromatographings necessary. Six of the compounds isolated were identified as periplocymarin (S 1), cymarin (S 2), periplocin (S 4), k-strophanthin-β (S 5), erysimoside (S 7), and k-strophanthoside (S 8).

From the k-strophanthoside by hydrolysis under mild conditions (0.05 N H_2SO_4) we obtained strophanthotriose and strophanthidin; the exhaustive methylation of the strophanthotriose and subsequent hydrolysis gave 2,3,4,6-tetra-O-methyl-D-glucose, cymarose, and 2,3,4-tri-O-methyl-D-glucose; the partial acid hydrolysis of strophanthoside with 0.1 N sulfuric acid gave gentiobiose (6-O-β-D-glucopyranosyl-D-glucose), in addition to strophanthotriose, strophanthobiose, D-glucose, and D-cymarose. The enzymatic hydrolysis of strophanthoside yielded cymarin, k-strophanthin-β, and D-glucose. These facts confirm the structure of k-strophanthoside (see [2, 10-13]) as strophanthidin 3β-[O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranoside] (I).

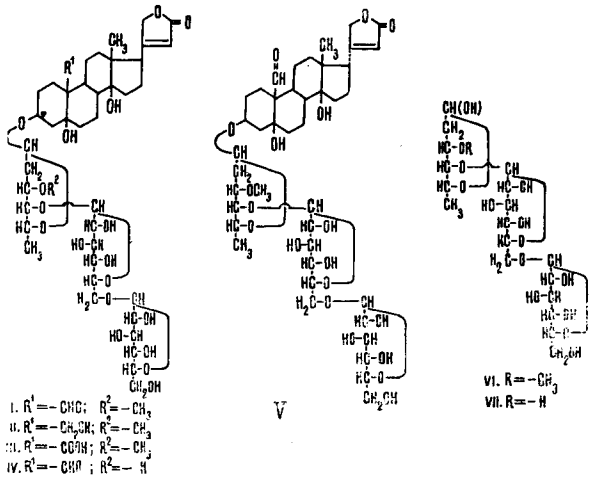
Cardenolide S 9 has the composition $C_{42}H_{66}O_{19}$, corresponding to a steroid triglycoside. Its UV spectrum is characterized by one absorption maximum at 218 nm (log ε 4.16) due to a butenolide ring. The glycoside is readily hydrolyzed by 0.05 N sulfuric acid, forming strophanthidol and strophanthotriose. The reduction of k-strophanthoside with sodium borohydride forms a glycoside identical with S 9. According to these facts, cardenolide S 9 is strophanthidol 3β-[O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranoside] (II). (See scheme on next page.)

In view of the fact that the product of the reduction of k-strophanthoside has been described previously [6] and has been given the trivial name of "k-strophanthol-γ" we have also adopted this name for the natural glycoside S 9.

Substance S 10 has the composition $C_{41}H_{62}O_{19}$. Its UV spectrum shows an absorption maximum at 218 nm (log ε 4.16) and a shoulder in the ~300 nm region (log ε ~ 1.9), which are due to a butenolide ring

* For Communication I, see [1].

Khar'kov Scientific-Research Institute of Pharmaceutical Chemistry. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 180-188, March-April, 1972. Original article submitted September 14, 1971.



and to a carbonyl group. Under the influence of an enzyme preparation from the grape snail, S 10 hydrolyzes to the monoglycoside erysimin and D-glucose, while on brief enzymatic hydrolysis erysimoside is formed, in addition to the products already mentioned. As is well known [14], erysimoside is strophanthidin 3β-[O-β-D-glucopyranosyl-(1→4)-β-digitoxopyranoside]. One would think the results obtained indicate that S 10 is nothing other than glucoerysimoside, i.e., the very glycoside that was detected for the first time in the plant under consideration by Kaiser et al. [8]. However, the results of a direct comparison of S 10 with glucoerysimoside isolated by us previously [15] from Cheiranthus allioni hort. show that these substances are different. The structure of the glucoerysimoside from Cheiranthus has been established; it is strophanthidin 3β-[O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-β-D-digitoxopyranoside]. The assumption that the substances mentioned differ in the position of attachment of the terminal D-glucose was confirmed by the investigations performed.

The hydrolysis of S 10 under mild conditions gave the aglycone strophanthidin and a trisaccharide with the composition $C_{18}H_{32}O_{14}$ which was named, after its structure had been established, gentiobiosyl-digitoxose. The partial hydrolysis of S 10 with 0.1 N sulfuric acid gave – in addition to the trisaccharide – gentiobiose, D-glucose, D-digitoxose, and digilanidobiose. Methylation of the trisaccharide and subsequent hydrolysis gave 2,3,4,6-tetra-O-methyl-D-glucose, D-cymarose, and 2,3,4-tri-O-methyl-D-glucose, namely the same products that are formed in the methylation and hydrolysis of strophanthotriose. The facts given show that in the glycoside S 10 all the monosaccharides are present in the form of pyranoses and are linked linearly; the D-digitoxose is attached to the aglycone (at C_3), D-glucose occupies the middle position, at C_4 of the digitoxose, and the terminal D-glucose is attached to the preceding one at C_6. An analysis of molecular rotations in accordance with Klyne's rule [9] shows that the monosaccharides are connected by β-glycosidic bonds.

Thus, glycoside S 10 is strophanthidin 3β-[O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-β-D-digitoxopyranoside] (IV), and the trisaccharide obtained from it is O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-β-D-digitoxose (VII). It will apparently be desirable to call glycoside S 10 neoglucoerysimoside, since the name glucoerysimoside has become established in the special and handbook literature for a compound of different structure (see above).

Cardenolide S 11, which, after its structure had been established, was called 17βH-strophanthoside, is a triglycoside with the composition $C_{42}H_{64}O_{19}$. Its UV spectrum has an absorption maximum at 218 nm (log ε 4.19) and a shoulder in the ~300 nm region (log ε≈ 1.9) due to a butenolide ring and a carbonyl group. The hydrolysis of the glycoside gave strophanthotriose and an aglycone identified as 17βH-strophanthidin [17, 18]. In view of the fact that the structure of strophanthotriose has been established (see above), this glycoside can be characterized as 17βH-strophanthidin 3β-[O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranoside], and the structure is represented by formula V.

Cardenolide S 12 is a triglycoside with the composition $C_{42}H_{64}O_{20}$. Its UV spectrum has an absorption maximum at 219 nm (log ε 4.19). The acid hydrolysis of the glycoside with 0.05 N sulfuric acid gave strophanthotriose and strophanthidin-19-carboxylic acid [16]. On the basis of these facts and the structure of strophanthotriose, the glycoside can be characterized as strophanthoside-19-carboxylic acid (III). A confirmation of the correctness of this structure (III) is the fact that the oxidation of k-strophanthoside with potassium permanganate forms a polar cardenolide chromatographically identical with the glycoside S 12.

The determination of the structures of cardenolides S 3 and S 6 will be reported separately. Only some of their properties are given in the present paper.

EXPERIMENTAL

For analysis, the substances were dried at 80°C in a vacuum of 0.01 mm Hg over phosphorus pentoxide for 4 h. The paper chromatography of the compounds under investigation and the determination of their relative polarities in the form of P_M were performed in the following solvent systems: 1) toluene–butan-1-ol (1:2)/water; 2) methyl ethyl ketone–m-xylene (1:1)/formamide; 3) butan-1-ol–acetic acid (4:1)/water; 4) benzene–methyl ethyl ketone (1:1)/water; and 5) benzene/formamide. All the glycosides isolated gave positive Legal and Raymond reactions. The following abbreviations are used in the text: k-s-β – k-strophanthin-β; TMG – tetra-O-methyl-D-glucose; G – D-glucose.

The Strophanthus seeds, after comminution and defatting with petroleum ether, were exhaustively extracted with 96% and 70% ethanol. The extract was concentrated in vacuum to an aqueous residue. The aqueous solution was additionally purified with petroleum ether, and then with alumina. The glycosides were extracted three times with chloroform–ethanol (2:1), after which the aqueous phase was saturated with sodium sulfate and treated with the same solvent until the cardenolide reactions were negative. The ethanolic-chloroformic solutions were dried over anhydrous sodium sulfate and evaporated in vacuum. The total glycosides obtained were separated by partition chromatography in columns of silica gel or cellulose, using the toluene–butan-1-ol (4:1 to 1:3)/water systems. The ratio of the mixture of glycosides being separated to the dry support was 1:200, or, in individual cases, 1:400.

Cymarin (S 2). The glycoside was crystallized from methanol; mp 142-143°C, $[\alpha]_D^{22}+36.3 \pm 2°$ (c 1.10; chloroform). It gave a positive Keller-Kiliani reaction. With conc. H_2SO_4 it formed the following time-dependent colorations: 0 min – cinnamon brown; 5 min – cinnamon. A mixed sample with authentic cymarin showed no depression of the melting point (142-144°C). Paper chromatography also showed the identity of these substances.

Periplocymarin (S 1). The substance was crystallized from methanol–ether; mp 138-141°C, $[\alpha]_D^{24}+28.3 \pm 3°$ (c 0.72; chloroform). The Keller-Kiliani reaction was positive. With conc. H_2SO_4 it formed the following colorations: 0 min – cinnamon; 25 min – blue; 90 min – blue-green.

The glycoside (10 mg) was dissolved in 5 ml of ethanol, 5 ml of 0.1 N sulfuric acid was added, and the mixture was heated at 90°C for 30 min. Then the solution was neutralized with barium carbonate, filtered, and evaporated. Paper chromatography of the residue showed the presence of periplogenin and D-cymarose.

Cardenolide S 3. The substance crystallized from ethanol in the form of large prisms; mp 288-289°C; $[\alpha]_D^{22}+63.2 \pm 3°$ [c 0.60; chloroform–methanol–pyridine (1:1:0.5)]. The Keller-Kiliani reaction (for the presence of 2-deoxysugars) and the reaction with tetranitromethane (for an isolated C=C) were negative. With conc. H_2SO_4 it formed the following colorations: 0 min – orange; 5 min – red; 220 min – crimson-red. UV spectrum: $\lambda_{max}^{ethanol}$ 218 and 285 nm.

Periplocin (S 4). The glycoside crystallized from water, mp 205-208°C, $[\alpha]_D^{25}+22.3 \pm 2°$ (c 1.00; methanol). With conc. H_2SO_4 it formed the following colorations: 0 min – cinnamon; 5 min – blue; 90 min – blue-green. A solution of 10 mg of the glycoside in 0.1 ml of dimethylformamide was treated with 10 mg of an enzyme preparation from the grape snail dissolved in 1 ml of water, and the mixture was left at 42°C for 24 h. The enzymes were precipitated with hot ethanol, and the solution was evaporated. According to paper chromatography, the residue consisted of periplocymarin and D-glucose.

k-Strophanthin-β (S 5). The glycoside crystallized from water; mp 227-232°C, $[\alpha]_D^{23}+28.7 \pm 3°$ (c 1.00; methanol). With conc. H_2SO_4 it formed the following colorations: 0 min – green; 2 h – yellow; 7 h – light brown. A mixed melting point of S 4 with a sample of k-strophanthin-β and paper chromatography showed their identity.

Cardenolide S 6. The substance crystallized from isopropanol; mp 182-184°C, $[\alpha]_D^{23}+26.1 \pm 2°$ (c 1.00; ethanol). On paper chromatography in solvent system 1, $R_{k-s-\beta}$ 0.37. With conc. H_2SO_4 it formed the following colorations: 0 min – brown; 3 min – red; 15 min – crimson-red; 120 min – red with a violet tinge.

Erysimoside (S 7). The substance crystallized from ethanol; mp 235-241°C, $[\alpha]_D^{23}$ + 19.0 ± 2° (c 1.12; methanol). With conc. H_2SO_4 it formed the following colorations: 0 min – green; 30 min – brown. UV spectrum; $\lambda_{max}^{ethanol}$ 219 and 305 nm (log ε 4.17; 1.51). A mixture with a sample of erysimoside showed no depression of the melting point (235-241°C). On paper chromatography, these substances appeared at the same level.

k-Strophanthoside (S 8). The glycoside crystallized from butan-1-ol; mp 179-182°C, $[\alpha]_D^{25}$ + 12.7 ± 2° (c 1.10; ethanol). With conc. H_2SO_4 it formed the following colorations: 0 min – green; 10 min – brown; 85 min – lemon-yellow; 105 min – light green. On paper chromatography in solvent system 1, $R_{k-S-\beta}$ 0.22. The molecular weight of 874.7 found (by the spectrophotometric method) and the results of elementary analysis correspond to the composition $C_{42}H_{64}O_{19}$. UV spectrum: $\lambda_{max}^{ethanol}$ 217 nm (log ε 4.16) with a shoulder in the ~300 nm region (log ε 1.87).

Strophanthotriose and Strophanthidin. A solution of 0.8 g of strophanthoside in 50 ml of 0.05 N sulfuric acid was heated at 80°C for 40 min. The aglycone was extracted from the cooled solution with a mixture of chloroform and propan-1-ol (5 : 1; 5 × 50 ml). The combined alcoholic-chloroformic extracts were treated with a 2 N solution of sodium carbonate (15 ml) and with water (4 × 10 ml) and were evaporated. The residue, which consisted of the aglycone, was crystallized from ethanol. The aglycone melted at 140-144°C/228-232°C; $[\alpha]_D^{25}$ + 44.7 ± 2° (c 1.00; methanol). With conc. H_2SO_4 it formed the following colorations: 0 min – yellow-green; 5 min – greenish-orange; 25 min – green. On paper chromatography it had the same R_f values as a sample of strophanthidin.

The aqueous solution containing the sugar component was neutralized with barium carbonate, filtered through a layer of kieselguhr, and concentrated in vacuum to a syrupy residue. After the addition of absolute ethanol, crystals of strophanthotriose (0.4 g) deposited. The recrystallized strophanthotriose had mp 217-223°C; $[\alpha]_D^{25}$ + 7.7 ± 2° (c 1.00; water; measurement after equilibrium had become established). In solvent system 3, R_G 0.51. The elementary analysis corresponded to the formula $C_{19}H_{34}O_{14}$.

Methylation of Strophanthotriose. Hydrolysis of Methylated Strophanthotriose and Identification of the Hydrolysis Products. With heating, 0.2 g of strophanthotriose was dissolved in 15 ml of dimethylformamide, and 4 ml of methyl iodide and 8 g of freshly prepared silver oxide were added. The reaction mixture was heated with constant stirring for 11 h. During the reaction, a further 7 ml of ethyl iodide and 10 g of silver oxide were added in small portions. The mixture was diluted with 50 ml of chloroform, and the precipitate was filtered off and washed with chloroform, and the filtrate was evaporated in vacuum. The reaction product was methylated again similarly.

The methylated trisaccharide was dissolved with heating in 35 ml of acetic acid, and 55 ml of hot water and 10 ml of conc. hydrochloric acid were added. The mixture was heated in the boiling water bath for 1 h. The cooled solution was neutralized with sodium carbonate to pH 5 and was treated with chloroform–ethanol (2 : 1 ; 5 × 50 ml). The ethanolic-chloroformic extract was washed with 15 ml of water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was chromatographed on 10 g of alumina (activity grade III). It was eluted with chloroform and then with chloroform–ethanol (95 : 5 to 65 : 35). This gave two monosaccharides. One of them (1) had mp 90-95°C (diethyl ether), $[\alpha]_D^{23}$ + 83.3 ± 5° (c 0.53; methanol; after the equilibrium had become established). A mixture of sugar 1 and 2,3,4,6-tetra-O-methyl-D-glucose gave no depression of the melting point (90-95°C). Paper chromatography also showed the identity of these substances.

Sugar 2 was obtained in the form of a viscous syrup, $[\alpha]_D^{24}$ + 66.8 ± 3° (c 1.72; ethanol). In solvent system 4 it had R_{TMG} 0.20. It was chromatographically identical with a sample of 2,3,4-tri-O-methyl-D-glucose. The separation of the methylated sugars also gave D-cymarose (traces). Apparently, the bulk of it decomposed during hydrolysis.

Partial Enzymatic Hydrolysis of k-Strophanthoside. A solution of 0.24 g of k-strophanthoside and 0.3 g of snail enzyme preparation in 20 ml of water was kept at 25°C for 45 min. The reaction was stopped by precipitating the enzyme with 200 ml of hot ethanol. The precipitate was filtered off. The solution was concentrated to a volume of about 5 ml and was diluted with 15 ml of water, and the glycosides were extracted with chloroform–ethanol (2 : 1; 5 × 30 ml). The extract was evaporated and the residue was chromatographed on 10 g of alumina (activity grade III). Mixtures of chloroform and ethanol (97 : 3 to 80 : 20) were used as the mobile phase. The first eluates yielded cymarin (mp 141-143°C), and the later ones gave k-strophanthin-β (mp 226-232°C; $[\alpha]_D^{24}$ + 29.2 ± 3°). Both glycosides were also identified by comparison with authentic samples.

Gentiobiose. k-Strophanthoside (0.4 g) was hydrolyzed with 0.1 N sulfuric acid (45 ml) in the boiling water bath for 3 h. The solution was neutralized with barium carbonate, filtered through kieselguhr, and evaporated. According to paper chromatography, the residue consisted of D-glucose, gentiobiose, strophanthobiose, D-cymarose, and strophanthotriose. The mixture of sugars was separated on a column of 80 g of cellulose in the butan-1-ol–acetic acid (4 : 1)/water system. After the passage of 200 ml of this mixture of solvents through the column, it was "cut" into 25 equal sections. Gentiobiose was obtained in the pure state from the zones containing the most polar sugar.

The gentiobiose crystallized from ethanol; mp 189-195°C; $[\alpha]_D^{25}+9.2\pm4°$ (c 0.48; water; after equilibrium had become established). Its elementary analysis corresponded to the composition $C_{12}H_{22}O_{11}$. In solvent system 3, R_G 0.45. On enzymatic hydrolysis it gave D-glucose (identified by paper chromatography). The properties described agree with those given in the literature [19].

k-Strophanthol-γ (S 9). The glycoside crystallized from isopropanol–ether; mp 192-195°C, $[\alpha]_D^{24}+8.0\pm2°$ (c 1.00; methanol). UV spectrum: $\lambda_{max}^{ethanol}$ 218 nm (log ε 4.16). In solvent system 1, $R_{k-s-\beta}$ 0.14. The elementary analysis corresponded to the composition $C_{42}H_{66}O_{19}$. With conc. H_2SO_4 it formed the following colorations: 0 min – yellow; 1 min – brown; 135 min – brown-blue.

The glycoside (80 mg) was hydrolyzed with 0.05 N sulfuric acid (10 ml) in a similar manner to k-strophanthoside (see above), and the aglycone [mp 139-142°C, $[\alpha]_D^{22}+36.1\pm3°$ (c 0.82; methanol)] and a trisaccharide (mp 217-222°C) were obtained in the individual states and were identified as strophanthidol and strophanthotriose by direct comparison with authentic samples.

k-Strophanthoside (5 mg) was dissolved in 0.3 ml of 90% ethanol, 3 mg of sodium tetrahydroborate was added, and the mixture was left for 15 min. Paper chromatography showed that the reduced product was identical with glycoside S 9.

Neoglucoerysimoside (S 10). The glycoside crystallized from butan-1-ol; mp 195-199°C; $[\alpha]_D^{25}+8.9\pm3°$ (c 0.83; ethanol). The results of elementary analysis agreed with the calculations for the composition $C_{41}H_{62}O_{19}$. UV spectrum: $\lambda_{max}^{ethanol}$ 218 nm (log ε 4.17) and a shoulder in the ~300 nm region (log ε ~ 1.9). On paper chromatography in system 1, the substance had $R_{k-s-\beta}$ 0.15. With conc. H_2SO_4 it gave the following coloration: 0 min – green; 2 min – brown; 150 min – lemon-yellow.

The partial enzymatic hydrolysis of 0.1 g of neoglucoerysimoside was performed just as for strophanthoside, except that the reaction time was 25 min. Erysimin (mp 150-154°C), erysimoside (mp 234-240°C), and D-glucose were isolated, and were identified by a direct comparison with authentic samples of these substances.

Gentiobiosyldigitoxose. Glycoside S 10 (0.26 g) was hydrolyzed with 0.05 N H_2SO_4 (20 ml) as described for k-strophanthoside. After similar working up of the hydrolysate (see above), the aglycone strophanthidin was isolated in the pure state with mp 139-142°C/227-232°C; $[\alpha]_D^{26}+45.2\pm3°$ (c 1.00; methanol), and so was a trisaccharide (120 mg), which we have called gentiobiosyldigitoxose.

Gentiobiosyldigitoxose crystallized from ethanol, mp 212-215°C, $[\alpha]_D^{24}+4.1\pm3°$ (c 0.44; water; after equilibrium had become established). In solvent system 3 it had R_G 0.33. The results of elementary analysis agreed with the calculated figures for the composition $C_{18}H_{32}O_{14}$. After enzymatic hydrolysis, performed on the micro scale, D-glucose and D-digitoxose (identified by paper chromatography) were detected.

Gentiobiosyldigitoxose (40 mg) was methylated with methyl iodide in the presence of silver oxide according to Kuhn [20] (compare the method described above), and after hydrolysis of the methylated product, 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-glucose (identified by paper chromatography) were obtained.

Gentiobiosyldigitoxose (10 mg) was hydrolyzed with 0.1 N H_2SO_4 (0.5 ml) in a sealed tube in the boiling water bath for 2.5 h. The solution was freed from acid and analyzed by paper chromatography. The following sugars were identified: gentiobiose, D-glucose, D-digitoxose, digilanidobiose, and the initial gentiobiosyldigitoxose.

17βH-Strophanthoside (S 11). The glycoside was crystallized from moist butanol, mp 176-179°C, $[\alpha]_D^{24}+6.4\pm3°$ (c 0.83; ethanol). UV spectrum: $\lambda_{max}^{ethanol}$ 218 nm (log ε 4.19) and a shoulder in the ~300 nm region (log ε ~ 1.9). With conc. H_2SO_4 it gave the following colorations: 0 min – green-brown; 1 min – brown; 50 min – brown-green. In solvent system 1, $R_{k-s-\beta}$ 0.17. The results of elementary analysis corresponded to the figures calculated for the composition $C_{42}H_{64}O_{19}$.

The glycoside (50 mg) was hydrolyzed with 0.05 N sulfuric acid in the same way as in the preceding cases. The appropriate working up of the hydrolysate gave the aglycone [mp 263-268°C, $[\alpha]_D^{25}+37.0\pm3°$ (c 0.72; methanol)] and a trisaccharide (mp 217-222°C), which were identified as 17β-strophanthidin and strophanthotriose. The identification was performed by comparison with samples of these substances obtained previously.

Strophanthoside-19-carboxylic Acid (S 12). The substance, with the composition $C_{42}H_{64}O_{20}$, crystallized from butanol–acetone; mp 195-198°C; $[\alpha]_D^{24}+21.1\pm3°$ (c 0.98; ethanol). UV spectrum: $\lambda_{max}^{ethanol}$ 219 nm (log ε 4.19). In solvent system 1, $R_{k-s-\beta}$ 0.05. On dissolution in conc. H_2SO_4 it gave the following colorations: 0 min – green-brown; 2 min – red; 90 min – red-violet.

The glycoside (0.3 g) was hydrolyzed in 40 ml of 0.05 N H_2SO_4 as described above. The aglycone was extracted from the hydrolysate with chloroform–propan-1-ol (2 : 1; 5 × 50 ml). The alcoholic-chloroformic extract was treated with 2 N sodium carbonate solution (20 ml). The sodium carbonate extract, into which practically the whole of the aglycone passed, was acidified with 1 N sulfuric acid. The cardenolide was extracted with chloroform–propan-1-ol (2 : 1; 5 × 60 ml). The alcoholic-chloroformic solution was washed with water (4 × 10 ml) and evaporated. The residue, which consisted of strophanthidin-19-carboxylic acid, was crystallized from acetone–diethyl ether–water; it had isomorphic forms: large prisms with mp 163-165°C and thin plates with mp 187-191°C; $[\alpha]_D^{25}+57.1\pm3°$ (c 0.86; ethanol). With conc. H_2SO_4 the aglycone gave the following colorations: 0 min – yellow-orange; 1 min – crimson-red; 120 min – lilac.

The aqueous solution of the hydrolysate, after the elimination of acid and crystallization from ethanol gave pure strophanthotriose with mp 217-223°C; after the establishment of equilibrium $[\alpha]_D^{24}+7.8\pm2°$ (c 1.20; water).

k-Strophanthoside (5 mg) was dissolved with heating in 1 ml of ethanol, 0.3 ml of a 0.2% acetone solution of potassium permanganate was added, and the mixture was left at 23°C for 15 min. Paper chromatography showed that the oxidized cardenolide so formed (it also contained the initial glycoside as an impurity) was identical with S 12.

SUMMARY

The seeds of Strophanthus kombe Oliv. have yielded 12 cardiac glycosides. Of them, six were identified with known cardenolides: S 1 – periplocymarin; S 2 – cymarin; S 4 – periplocin; S 5 – k-strophanthin-β; S 7 – erysimoside; S 8 – k-strophanthoside. The structure of k-strophanthoside as strophanthidin 3β-[O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranoside] has been confirmed.

This is apparently the first time that the other six glycosides have been isolated from Strophanthus kombe in the pure state. Cardenolides S 9, S 11, and S 12 have strophanthotriose as the carbohydrate component, and their aglycones are, respectively, strophanthidol, strophanthidin-19-carboxylic acid, and 17β H-strophanthidin. These glycosides have been named in accordance with the structures established for them: k-strophanthol-γ (S 9), strophanthoside-19-carboxylic acid (S 12) and 17βH-strophanthoside (S 11). Cardenolide S 10, which has been called neoglucoerysimoside, is strophanthidin 3β-[O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-β-D-digitoxopyranoside]. From neoglucoerysimoside a new trisaccharide has been obtained which we have called gentiobiosyldigitoxose. The structure of the latter can be characterized as O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-D-digitoxose.

LITERATURE CITED

1. I. F. Makarevich, Khim. Prirodn. Soedin., 508 (1969).
2. A. Stoll, J. Renz, and W. Kreis, Helv. Chim. Acta, 20, 1484 (1937).
3. W. A. Jacobs and A. Hoffmann, J. Biol. Chem., 67, 609 (1926).
4. R. Zelnik, J. V. Euw, O. Schindler, and T. Reichstein, Helv. Chim. Acta, 43, 593 (1960).
5. W. Blome, A. Katz, and T. Reichstein, Pharmac. Acta Helv., 21, 325 (1946).
6. E. Rabald and J. Kraus, Z. Physiol. Chem., 262, 39 (1940).
7. A. Katz and T. Reichstein, Helv. Chim. Acta, 28, 476 (1945).
8. F. Kaiser, E. Haack, N. Dölberg, and H. Spingler, Ann. Chem., 643, 192 (1961).
9. W. Klyne, Biochem. J., 47, No. 4 (1950).

10. H. Lichti and A. Wartburg, Helv. Chim. Acta, 44, 238 (1961).
11. W. A. Jacobs and A. Hoffmann, J. Biol. Chem., 69, 153 (1926).
12. C. Tamm, Fortschr. Chem. Organ. Naturstoffe, 13, 137 (1956).
13. J. C. Hess, A. Hunger, and T. Reichstein, Helv. Chim. Acta, 35, 2202 (1952).
14. V. A. Maslennikova, F. S. Khristulas, and N. K. Abubakirov, Dokl. Akad. Nauk SSSR, 124, 822 (1959); Zh. Obshch. Khim., 31, 2424 (1961).
15. I. F. Makarevich, Khim. Prirodn. Soedin., 566 (1970).
16. W. A. Jacobs, J. Biol. Chem., 57, 553 (1923); 74, 795 (1927).
17. W. A. Jacobs, J. Biol. Chem., 88, 519 (1930).
18. A. Katz and T. Reichstein, Pharm. Acta Helv., 19, 231 (1944).
19. I. Heilbron, Dictionary of Organic Compounds, 2nd ed., Eyre and Spottiswoode, London (1943-4).
20. R. Kuhn, H. Trischman, and J. Löw, Angew. Chem., 67, 32 (1955).