CARDIAC GLYCOSIDES FROM *Strophanthus kombe*

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Twelve cardiac glycosides and aglycons were isolated from Strophanthus kombe *seeds. Of these, eight were identified as cymarin, K-strophanthin-β, K-strophanthoside, periplocymarin, 17α-strophadogenin, erysimin (= helveticoside), erysimoside, and neoglucoerysimoside. Four glycosides, preliminarily designated Sk-x, Sk-y, Sk-z, and Sk-20, were new. Their chemical structures were established as 3*β-O-β-*D-glucopyranosyl-5*β*,14*β*,16*β*-trihydroxy-19-oxo-17*α*-card-20(22)enolide (17*α*-strophadogenin-3*-O-β-*D-glucoside), 3*β-O-β-*D-cymaropyranosyl-5*β*,14*β*,16*β*-trihydroxy-19-oxo-17*α*-card-20(22)enolide (17*α*-strophadogenin-3*-O-β-*D-cymaroside), 3*β-O-β-*D-cymaropyranosyl-4′*-O-β-*D-glucopyranosyl-6″*-O-β-*D-glucopyranosyl-5*β*,14*β*,16*β*-trihydroxy-19-oxo-17*α*-card-20(22)enolide (17*α*-strophadogenin-3*-O-strophanthotrioside), and 3*-O-β-*D-digitoxopyranosyl-4′*-O-β-*D-glucopyranosyl-6″*-O-β-*D-glucopyranosyl-5*β*,14*β*,19-trihydroxy-card-20(22)enolide (strophanthidol-3*-O-*gentiobiosyldigitoxoside), respectively.*

Key words: cardenolides, cardenolide glycosides, *Strophanthus kombe*, separation, new cardenolide glycosides, 17*α*-strophadogenin-3-*O*-*β*-D-glucoside, 17*α*-strophadogenin-3-*O*-*β*-D-cymaroside, 17*α*-strophadogenin-3-*O*-strophanthotrioside, strophanthidol-3-*O*-gentiobiosyldigitoxoside.

Strophanthus kombe Oliv. is a very important source of cardiac glycosides owing to their high content in the seeds of this plant. The plant is a bushy vine from tropical eastern Africa. The purified total cardioglycosides from *S. kombe* seeds are widely used as an injectable solution under the name strophanthin K in cardiology for treatment of cardiac deficiencies.

The chemical composition of *S. kombe* glycosides has been studied by many groups, including one of us [1-4]. Until the research presented herein, 17 cardiac glycosides and aglycons had been isolated from the plant: K-strophanthin-*β* [5-7], cymarin [5, 8], cymarol [8, 9], periplocymarin [8], emicymarin [8], 17*α*-emicymarin [8], erysimin (helveticoside) [8, 10], 17*α*-helveticoside [10], erysimoside [10], erysimosol [10], neoglucoerysimoside [2], K-strophanthoside [2, 7], K-strophanthol-*γ* [2], strophanthoside-19-carboxylic acid [2], 17*α*-strophadogenin [4], 3-epistrophanthidin [1, 3], and 3-epistrophanthidol [1, 3]. We established previously that *S. kombe* contains the free native aglycon 17*α*-strophadogenin, which has the complicated structure 3*β*,5*β*,14*β*,16*β*-tetrahydroxy-19-oxo-17*α*-card-20(22)enolide [4]. The presence of the free algycon in the plant suggests that glycosides of this aglycon are also present.

We intended to find namely this group of glycosides. They are interesting because they are new compounds and glycosides containing 17*α*-strophadogenin have not been obtained previously. The search was simplified by the fact that cardenolides and cardenolide glycosides with a C-16 OH group are developed on chromatograms by not only Raymond reagent but also so-called Jensen reagent [2, 11], which gives a spot that fluoresces intensely blue in UV light. Another group of cardenolides that occurs in strophanthus, 17*α*-cardenolides, also gives blue fluorescence with Jensen reagent (in UV light). However, their fluorescence is relatively weak.

We received purified total cardiosteroids of strophanthus, analyzed them chromatographically, and found that they consisted of at least 22 compounds, of which at least five were developed by Raymond and Jensen reagents.

Compounds were isolated pure using column chromatography over silica gel with elution by mixtures of $CHCl_3$:CH_3OH or $CHCl_3$:C_2H_5OH of increasing polarity. This afforded 12 pure cardiac glycosides and aglycons, 8 of which were identified as cymarin, periplocymarin, erysimin, K-strophanthin-*β*, erysimoside, K-strophanthoside, 17*α*-strophadogenin, and

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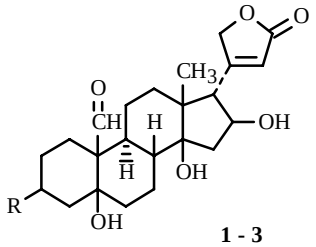
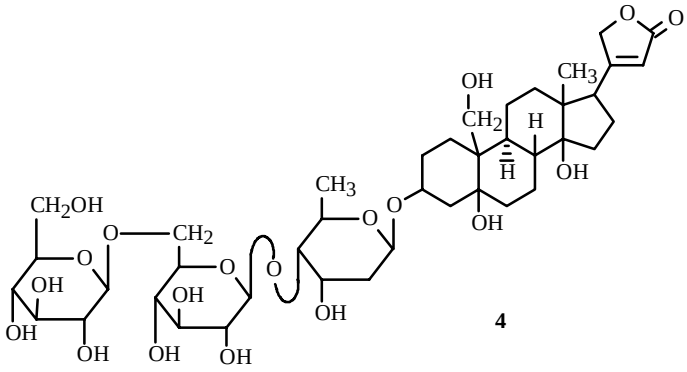
neoglucoerysimoside. They were identified using physicochemical properties and direct comparison with authentic samples (mixed melting points, TLC). Their chemical structures have been described [12].



1 - 3

1: R = β-D-Glc*p*
2: R = β-D-Cym*p*
3: R = β-D-Glc*p*(1→6)-β-D-Glc*p*(1→4)-β-D-Cym*p*



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Four glycosides that were preliminarily designated Sk-x (**1**), Sk-y (**2**), Sk-z (**3**), and Sk-20 (**4**) were new.

According to elemental analysis and hydrolysis products, we concluded that **1** was a monoglycoside of formula $C_{29}H_{42}O_{12}$. Assuming that **1** contained D-glucose (judging from the empirical formula), we carried out enzymatic hydrolysis, producing the pure algycon and a monosaccharide. The aglycon had the composition $C_{23}H_{32}O_7$. Its IR spectrum was characterized by absorption bands of a butenolide ring (1740 cm^{-1} C=O and 1630 cm^{-1} C=C of an unsaturated lactone ring), an aldehyde (1719 cm^{-1} C=O and 2780 cm^{-1} CH), and four OH groups (four well resolved bands at 3360, 3465, 3525, and 3570 cm^{-1}).

The aglycon was a 17α-cardenolide, which was demonstrated by an allomerization test consisting of heating in DMF in the presence of sodium tosylate and sodium acetate. It showed that the aglycon was unchanged. The physicochemical properties (melting point, specific rotation), allomerization data, and IR spectrum corresponded with those of 17α-strophadogenin. A direct comparison of the produced aglycon with 17α-strophadogenin confirmed that they were identical.

The monosaccharide was identified as glucose from the melting point (146-148°C) and chromatographic properties. The increment of molecular rotation of the carbohydrate part in **1** (70 ± 5°, calculated from the Klyne rule [13]) corresponded with the molecular rotation of a β-methyl-D-glucopyranoside (-66°), which was consistent with a β-glycoside bond and the pyranose form of the D-glucoside.

The completed studies showed that glycoside Sk-x was 17α-strophadogenin-3β-*O*-β-D-glucopyranoside or (full chemical name) 3β-*O*-β-D-glucopyranosyl-5β,14β,16β-trihydroxy-19-oxo-17α-card-20(22)enolide (**1**).

Compound Sk-z was a monoglycoside of formula $C_{30}H_{44}O_{10}$ according to elemental analysis and hydrolysis products. It contained a 2-deoxysugar (positive Keller-Kiliani reaction). Acid hydrolysis under mild conditions produced quantitatively the aglycon and monosaccharide.

The aglycon had the composition $C_{23}H_{32}O_7$. Acetylation formed a diacetate of formula $C_{27}H_{36}O_9$. An analysis of the course of the acetylation reaction revealed that one of the acetylated OH groups was typically axial whereas the other was equatorial or pseudoequatorial if it was located on C-16. A positive Jensen reaction and spectral data confirmed that the aglycon had a 16β-OH group. The PMR spectrum of the diacetate was more easily obtained than that of the starting aglycon because the former was soluble in solvents suitable for this. The PMR spectrum of the diacetate had the following characteristic signals: 1.12 ppm, 3H singlet, 18-CH_3; 1.94 ppm, 6H singlet, acetyl; 4.27 ppm, 1H doublet, 17-H; 4.90 ppm, 2H doublet, methylene of a butenolide ring; 5.20 ppm, 1H multiplet, 3α-H; 6.0 ppm, 1H doublet, 16α-H; 10.01 ppm, 1H singlet, CHO. These data correspond with the PMR spectrum of 17α-strophadogenin diacetate, which we described previously [4].

In general, all data including direct comparison with an authentic sample indicated that the aglycon of the glycoside Sk-z was 17α-strophadogenin.

The monosaccharide was a 2-deoxysugar (positive Keller—Kiliani reaction) and gave a paper chromatogram identical to that of D-cymarose.

The $[M]_D$ of cymarose in Sk-z was +19 ± 4°, which was consistent with the $[M]_D$ of β-D-cymaropyranose in the known glycoside cymarin (+18 ± 4°). This suggested that the D-cymarose in the glycoside under discussion was bound by a β-glycoside bond and adopted the pyranose form.

Thus, Sk-z was 17α-strophadogenin-3-*O*-β-D-cymaroside or (full chemical name) 3β-*O*-β-D-cymaropyranosyl-5β,14β,16β-trihydroxy-19-oxo-17α-card-20(22)enolide (**2**).

Elemental analysis and hydrolysis products indicated that Sk-y was a triglycoside of formula $C_{42}H_{64}O_{20}$. Enzymatic hydrolysis formed a monoglycoside and a monosaccharide. According to its properties and a direct comparison with a sample, the monoglycoside was identical to the aforementioned 17α-strophadogenin-3-*O*-β-D-cymaropyranoside (**2**). The monosaccharide was identified by paper chromatography (PC) as D-glucose.

The presence in Sk-y of a 2-deoxysugar (D-cymarose) bonded directly to the aglycon enabled acid hydrolysis to be carried out under mild conditions to produce the aglycon and a trisaccharide. The properties of the aglycon were identical to those of 17α-strophadogenin. A comparison with a sample of this aglycon on TLC and a mixed melting point confirmed that they were identical. The trisaccharide was identified by direct comparison with a sample as a strophanthotriose.

Thus, the experimental results indicated unambiguously that Sk-y was a 17α-strophadogenin-3-*O*-strophanthotrioside or (full chemical name) 3β-*O*-β-D-cymaropyranosyl-4′-*O*-β-D-glucopyranosyl-6″-*O*-β-D-glucopyranosyl-5β,14β,16β-trihydroxy-19-oxo-17α-card-20(22)enolide (**3**).

Sk-20 was a triglycoside of formula $C_{41}H_{64}O_{19}$ accoding to elemental analysis and hydrolysis products. Enzymatic hydrolysis of Sk-20 produced the known monoglycoside helveticosol and D-glucose. Helveticosol was identified by its properties and direct comparison with a sample. It was strophanthidol-3β-*O*-β-D-digitoxopyranoside [11]. D-Glucose was identified using PC.

Considering that Sk-20 contained a 2-deoxysugar (D-digitoxose) bound directely to the aglycon, we performed acid hydrolysis under mild conditions to produce the aglycon and a trisaccharide. The properties of the aglycon were identical to those of strophanthidol. Direct comparison with a sample, including the IR spectrum, confirmed that they were identical.

The trisaccharide was identified using PC as gentiobiosyldigitoxose (direct comparison with a sample), which is 4-*O*-(β-D-glucopyranosyl-6′-*O*-β-D-glucopyranosyl)-D-digitoxose [2].

The experimental results for the identification of the acid and enzymatic hydrolysis products led unambiguously to the conclusion that Sk-20 was 3β-*O*-β-D-digitoxopyranosyl-4′-*O*-β-D-glucopyranosyl-6″-*O*-β-D-glucopyranosyl-5β,14β,19-trihydroxycard-20(22)e nolide (**4**) (strophanthidol-3-*O*-gentiobiosyldigitoxoside).

EXPERIMENTAL

Cardiosteroids were analyzed by TLC on Sorbfil plates using $CHCl_3$:CH_3OH:H_2O (85:15:0.7, 80:20:1, and 70:30:1). Sugars were analyzed by PC using *n*-butanol:methylethylketone:borate buffer (1:1:2). Borate buffer contained equal volumes of aqueous boric acid (0.1 M) and aqueous borax (0.1 M [14].

Elemental analysis was performed on an automated model 1106 C—H—N—S analyzer. Analyses of all compounds corresponded with those calculated. Melting points were determined on a Kofler block. IR spectra were recorded on a Specord-75 IR (KBr disks); PMR spectra, on a Varian VX-200 instrument. Seeds of strophanthus were graciously supplied by L′vov Chemical Pharmaceutical Plant.

Isolation of Cardiosteroids. Seeds of strophanthus (2 kg) were ground in a ball mill, defatted with Nefras, and extracted with ethanol (80°). The extract was evaporated in vacuo to give a thick extract that was dissolved in ethanol (70°, 200 mL), diluted with hot water (300 mL), cooled to room temperature, and purified with petroleum ether (2 × 200 mL).

Cardiosteroids were extracted from the aqueous alcohol solution by $CHCl_3$:alcohol (2:1, 5 × 300 mL). The upper layer was treated with NaCl (90 g) and extracted with the same solvent mixture (4 × 300 mL). After extraction nine times, the upper phase still showed a strong reaction for cardenolides so it was extracted again with water-saturated *n*-butanol (5 × 250 mL). The $CHCl_3$:alcohol and *n*-butanol extracts were evaporated separately in vacuo to afford polar and moderately polar cardiosteroids (No. 1, 63 g) and highly polar glycosides (No. 2, 27 g).

The compounds were separated over columns of KSK silica gel (0.05-0.1 mm fraction) using a 30-fold excess of silica gel relative to the total amount of compounds to be separated. The eluents were $CHCl_3$:C_2H_5OH and $CHCl_3$:CH_3OH mixtures of increasing polarity (99:1→70:30). Fractions (40 mL) were collected by an automated collector to produce the following compounds.

Cymarin, mp 185-187°C (ethanol), $[\alpha]_D^{20}$ +35 ± 2° (*c* 1.1, $CHCl_3$), $C_{30}H_{44}O_9$.

17α-Strophadogenin, mp 287-289°C (CH_3OH), $[\alpha]_D^{20}$ +64.9 ± 2° (*c* 1.0, CH_3OH:C_5H_5N), $C_{23}H_{32}O_7$.

Periplocymarin, mp 205-208°C (ethanol), $[\alpha]_D^{20}$ +30.1 ± 2° (*c* 0.9, CH_3OH), $C_{30}H_{46}O_8$.

Erysimin, mp 150-154°C (water-sat. EA), $[\alpha]_D^{20}$ +28.3 ± 2° (*c* 1.1, CH_3OH), $C_{29}H_{42}O_9$.

K-Strophanthin-β, mp 193-195°C (*i*-PrOH), $[\alpha]_D^{20}$ +32.0 ± 2° (*c* 1.2, CH_3OH), $C_{36}H_{54}O_{14}$.

Erysimoside, mp 171-173/237-241°C (*i*-PrOH), $[\alpha]_D^{20}$ +19.5 ± 2° (*c* 1.0, CH_3OH), $C_{35}H_{52}O_{14}$.

K-Strophanthoside, mp 177-182/198-200°C (*i*-PrOH), $[\alpha]_D^{20}$ +14.0 ± 2° (*c* 1.1, CH_3OH), $C_{42}H_{64}O_{19}$.

Neoglucoerysimoside, mp 196-199°C (*i*-PrOH), $[\alpha]_D^{20}$ +9.0 ± 2° (*c* 1.1, CH_3OH), $C_{41}H_{62}O_{19}$.

17α-Strophadogenin-3-*O*-β-D-glucoside (1), mp 209-212°C (*i*-PrOH), $[\alpha]_D^{20}$ +35.1 ± 2° (*c* 0.9, CH_3OH), $C_{29}H_{42}O_{12}$.

Enzymatic hydrolysis of 1 was carried out using enzyme preparation from pancreatic juice of grape snail [2] to produce the aglycon and monosaccharide.

Aglycon of 1 (17α-strophadogenin), mp 288-289°C (CH_3OH), $[\alpha]_D^{20}$ +65.0 ± 2° (*c* 0.85, CH_3OH:C_5H_5N), $C_{23}H_{32}O_7$ [4], mixed mp 288-289°C.

Monosaccharide of 1 (D-glucose), mp 146-148°C (ethanol:water). PC showed it was identical to an authentic sample.

17α-Strophadogenin-3-*O*-β-D-cymaroside (2), mp 221-223°C (ethanol), $[\alpha]_D^{21}$ +51.7 ± 2° (*c* 0.8, CH_3OH:C_5H_5N), $C_{30}H_{44}O_{10}$.

Acid Hydrolysis of 2. Glycoside (0.25 g) was dissolved in ethanol (15 mL), treated with H_2SO_4 solution (15 mL, 0.1 N), and boiled for 50 min. The alcohol was distilled. The precipitated crystalline aglycon was separated and recrystallized from CH_3OH.

Aglycon of 2, mp 288-289°C, $[\alpha]_D^{20}$ +64.8 ± 2° (*c* 0.8, CH_3OH:C_5H_5N), $C_{23}H_{32}O_7$. Acetylation with acetic anhydride in pyridine produced the diacetate of formula $C_{27}H_{36}O_9$, mp 212-215°C, $[\alpha]_D^{20}$ +98.0 ± 4° (*c* 0.4, CH_3OH). The PMR spectrum of the diacetate was identical to that described earlier for 17α-strophadogenin diacetate [4].

Monosaccharide of 2. The aqueous hydrolysate was neutralized with $BaCO_3$ and analyzed by PC. The resulting monosaccharide was identical to D-cymarose [12].

17α-Strophadogenin-3-*O*-strophanthotrioside (3), mp 183-185°C (ethanol), $[\alpha]_D^{20}$ +24.2 ± 2° (*c* 1.1, CH_3OH), $C_{42}H_{64}O_{20}$.

Enzymatic hydrolysis of the glycoside was performed as described above to produce a monoglycoside and monosaccharide.

Monoglycoside, mp 221-223°C (ethanol), $[\alpha]_D^{20}$ +51.4 ± 2° (*c* 1.0, CH_3OH). These properties correspond to those described above for new glycoside **2**. Mixed melting point with **2** (mp 221-223°C) and TLC analysis confirmed that they were identical.

Monosaccharide in the hydrolysate was identical by PC with D-glucose.

Acid hydrolysis of 3 was performed with H_2SO_4 (0.05 N) as described above for **2** to produce the aglycon and a trisaccharide.

Aglycon of 3 was 17α-strophadogenin.

Trisaccharide of 3 was identical by PC to a sample of strophanthotriose [2].

Strophanthidol-3-*O*-gentiobiosyldigitoxoside (4) was crystallized from CH_3OH:Et_2O, mp 117-120°C, $[\alpha]_D^{20}$ +10.0 ± 2° (*c* 1.0, CH_3OH), $C_{41}H_{64}O_{19}$.

Enzymatic hydrolysis of 4 was performed as usual (see above) to give a monoglycoside and monosaccharide.

Monoglycoside, mp 147-150°C (acetone), $[\alpha]_D^{21}$ +27.5 ± 2° (*c* 0.7, CH_3OH). Its properties were identical to those of helveticosol, which is strophanthidol-3β-*O*-β-D-digitoxoside [12]. Comparison of the monoglycoside with a sample of helveticosol confirmed that they were identical.

Monosaccharide produced by enzymatic hydrolysis was identical by chromatographic analysis to D-glucose.

Acid hydrolysis of 4 was performed with H_2SO_4 (0.05 N) as described above for **2** to produce the aglycon and trisaccharide.

Aglycon of 4 was crystallized from ethanol (50%), mp 140-142°C, $[\alpha]_D^{20}$ +36.8 ± 2° (*c* 0.5, CH_3OH). The IR spectrum of the resulting aglycon and its properties were identical to those of strophanthidol [11].

Trisaccharide of 4 was analyzed using PC. The results showed that the resulting sugar was identical to a sample of gentiobiosyldigitoxose [2].

REFERENCES

1. I. F. Markarevich, *Khim. Prir. Soedin.*, 508 (1969).
2. I. F. Makarevich, *Khim. Prir. Soedin.*, 180 (1972).
3. I. F. Makarevich, *Khim. Prir. Soedin.*, 57 (1970).
4. I. F. Makarevich, N. V. Kovchanko, Yu. I. Gubin, K. V. Zhuravlev, and T. V. Slyusarskaya, *Khim. Prir. Soedin.*, 724 (1993).
5. W. A. Jacobs and A. Hoffmann, *J. Biol. Chem.*, **67**, 153 (1926).
6. H. Lichti and A. Wartburg, *Helv. Chem. Acta*, **44**, 238 (1961).
7. A. Stoll, J. Renz, and W. Kreis, *Helv. Chem. Acta*, **20**, 1484 (1937).
8. R. Zelnik, J. V. Euw, O. Schindler, and T. Reichstein, *Helv. Chem. Acta*, **43**, 593 (1960).
9. W. Blome, A. Katz, and T. Reichstein, *Pharm. Acta Helv.*, **21**, 325 (1946).
10. F. Kaiser, E. Haack, N. Dolberg, and H. Spingler, *Liebigs Ann. Chem.*, **643**, 192 (1961).
11. K. B. Jensen and K. Tennoe, *J. Pharm. Pharmacol.*, **7**, 334 (1950).
12. L. T. Malaya, I. F. Makarevich, N. V. Kovchanko, and Yu. G. Gorb, *Cardiac Glycosides* [in Russian], Osnova, Khar′kov (1996).
13. W. Klyne, *Biochem. J.*, **47**, No. 4, xli (1950).
14. R. Tschesche, H. J. Wulff, U. Dolberg, and G. Snatzke, *Naturwissenschaften*, **46**, 109 (1959).