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CARDENOLIDES OF *Strophanthus kombe*
III. 17α-STROPHADOGENIN

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17α-Strophadogenin, which is 3β,5,14,16β-tetrahydroxy-19-oxo-5β,14β-card-17βH-20(22)-enolide has been obtained in the pure form for the first time and has been studied.

As reported previously [1], from the seeds of the strophanthus *Strophanthus kombe* Oliv., together with other compounds, a cardenolide has ben obtained, provisionally designated as S3, with properties (melting point, $[\alpha]_D$ differing from those of known aglycons. In view of these facts, we set ourselves the task of studying the properties of S3 in more detail and establishing its chemical structure. To establish its structure we have used both spectral and chemical methods.

S3 has the composition $C_{23}H_{32}O_7$. Its mass spectrum showed the presence of a weak signal of the protonated molecular ion with m/z 421 (0.5%; M+1). The splitting out of substituents from the molecular ion gave peaks of ions with m/z (%) 402 (1; M − H_2O), 384 (2; M − $2H_2O$), 374 (10; M − H_2O − CO), 366 (7; M − $3H_2O$), 356 (100; M − $2H_2O$ − CO), 348 (2; M − $4H_2O$, or M − H_2O − (CH_2=CH−CH=CH_2, sec [2]), 338 (10.6; M − $3H_2O$ − CO), 320 (10; M − $4H_2O$ − CO, or M − H_2O − butadiene − CO), 305 (5; M − $4H_2O$ − CO − CH_3).

In addition, there was the following series of main peaks 256 (5), 246 (3.5), 233 (5), 228 (6), 227 (5), 215 (8.5), 213 (8.4), 205 (9.5), 203 (12), 197 (8.4), 187 (21.3), 185 (13), 181 (26.5), 180 (29), 179 (40.8), 177 (47), 176 (75), 160 (51.2), 159 (49.5), 133 (47), 131 (15.3), 129 (15.3), 119 (15.4), 105 (30), 97 (25), 91 (41.8).

Judging from its mass spectrum, it was possible to assume the presence in S3 of four OH groups, including the possibility of the presence of a 3,5β-dihydroxy fragment, snd also an aldehyde group.

Spectral investigations confirmed the presence of an aldehyde group in the aglycon. In the UV region of the spectrum there were two absorption maxima, $\lambda_{max}^{ethanol}$: 218 nm, log ε 4.24 (butenolide ring) and 283 nm, log ε 2,25 (aldehyde group). In the IR spectrum there were two absorption bands, at 1719 and 2780 cm^{-1}, which are characteristic for an aldehyde group; the first of then is due to to the absorption of C=O, and the second to that of the C−H bond of the aldehyde group. Its presence was also shown by the oxidation of S3 with potassium permanganate, which led to the formation of a more polar cardenolide in the form of a carboxylic acid.

There is no isolated C=C double bond in the aglycon, as was shown by a negative reaction with tetranitromethane and by the PMR and IR spectra,

On interaction with acetic anhydride in pyridine, S3 formed a diacetate with the composition $C_{27}H_{36}O_9$. Analysis of the course of the acetylation of S3 by a method described previously showed that one of the OH groups undergoing acetylation is typically axial and the other equatorial, or pseudoequatorial if it occupies the 16β position.

S3 is a 17α-cardenolide. This was shown by the following factors. In the first place, in an allomerization test, consisting in heating the compound in DMF in the presence of sodium tosylate and sodium acetate, the aglycon underwent no change. Ordinary 17β-cardenolides isomerize under these conditionds into the more polar 17α-cardenolides. In the second place, the cardenolide under investigation was bioloically inactive or only weakly active; thus, it proved to be inactive in a dose of 0.90 mg/kg, which is characteristic for 17α-cardenolides.

On the basis of the IR spectra it was possible to deduce the presence of four OH groups in S3. There were four well-resolved bonds in the region of absorption of hydroxy groups, at 3360, 3465, 3525, and 3570 cm^{-1}.

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TABLE 1. Chemical Shifts of the Protons of Cardenolides in ^1H NMR Spectra

Compound	18-H_3	19-CHO	17-H	21-H_2	22-H	3-Hα	16-Hα	Other protons
Strophanthidin	1.00s	10.40 s	2.80 dd (9.6; 5.4)	5.00 dd (18.0; 1.8) 5.27 dd (18.0; 1.8)	6.13s	4.40m (W/2 8)		5.60 br.s s(OH); 6.00 s (OH)
17α-Stroph-anthidin	1.16s	10.45 s	3.40 t (9.6)	4.78 d (18.0) 4.94 dd (18.0; 1.8)	6.07s	4.42m (W/2 8)		5.68 br.s s(OH); 6.26s (OH)
S_3	1.24	10.46 s	3.74 d (8.4)	4.90 d (17) 5.04 d	6.27s	4.41m (W/2 10)	4.80 (W/2 20)	5.91 br. s s(OH); 6.20 br.s (OH)
S3 diacetate	1.22s	10.42 s	3.83 d (8.4)	4.90 d (15)	6.27s	5.30 m (W/2 10)	5.60 dt (2.0; 8.5)	1.94s (6H,AcO); 2.85 dd (9.6; 15.6 15-Hα-)

TABLE 2. Chemical Shifts of the Carbon Atoms of Cardenolides in the ^{13}C NMR Spectra

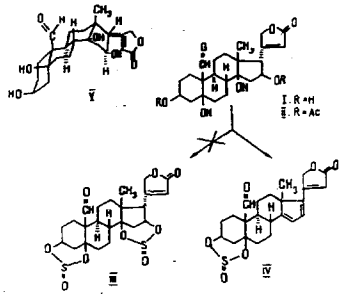
C atom	Strophanth-idin [6]	S_3	C atom	Strophanth-idin [6]	S_3
1	24.6	24.2 t	13	49.7	49.5 s
2	27.2	27.4 t	14	84.6	84.0 s
3	66.6	66.7 d	15	32.4	32.0 t
4	38.2	38.3 t	16	27.3	73.4 d
5	74.3	74.5 s	17	51.0	58.3 d
6	37.6	37.1 t	18	16.2	18.2 q
7	22.2	21.6 t	19	208.8	208.8 d
8	42.0	41.7 d	20	175.7	171.3 s
9	39.3	39.4 d	21	73.9	74.4 t
10	55.4	55.4 s	22	117.7	116.2 d
11	18.2	18.1 t	23	174.2	174.1 s
12	39.4	41.6 t			

Of the four OH groups two are secondary, being acetylatable under the usual conditions, and two tertiary and nonacetylatable. Assuming that the tertiary OH groups may occupy the "usual" positions for them at C-5 and C-14, and one of the seconday ones be located at C-3, it was necessary to determine the position of the fourth alcohol group and certain questions of the configuration of the substituents.

The optical rotatory dispersion spectrum of S3 had a negative Cotton effect, which showed the cis-linkage of rings A/B and, accordingly the β-configuration of the substituent at C-5.

When a chromatogram was revealed with the Jensen reagent (trichloroacetic acid in ethanol) the S3 spot fluoresced blue in UV light, which is characteristic for cardenolides containing an OH group at C-16 or a double C=C bond in the 16:17 position, and also for 17α-cardenolides. It is, however, true that 17α-cardenolides give only a weak blue fluorescence under these conditions.

On the basis of the experimental results obtained we assumed that the aglycon that we were studying could have structure (I), i.e., be 17α-strophadogenin, which has not hitherto been obtained in the pure form although its presence has been suggested in one of the glycosides isolated by N. K. Abubakirov et al. [4] and called 17α-gypsobioside [4].



N. K. Abubakirov kindly supplied us with a a mixture of two glycosides which we hydrolyzed under mild conditions and compared the reaction products with S3 chromatographically. It was found that one of the cardenolides present in the hydrolysate had a polarity close to that of S3 but was not identical with it. This complicated the situation and forced us to carry out additional experimental investigtions to establish the chemical structure of S3.

The presence or absence of a 16β-OH group can also be judged from the results of the chemical reaction with thionyl chloride. When two groupings (3β,5β-dihydroxy- and 14β,16β-dihydroxy-) are present one must expect the formation of the bisester (III). The reaction that we performed enabled us to obtain a mixture of compounds with a predominance of the anhydro product (IV). The cyclosulfite (IV) was isolated in the individual state with the aid of column chromatography on silica gel, It did not undergo acetylation, which indicated that it contained no secondary OH groups. Elementary analysis showed the composition $C_{23}H_{26}O_6S$, agreeing with structure (IV). The UV spectrum of (IV) reveaked two absorption maxima at $\lambda_{max}^{ethanol}$ 224 (log ε 4,31) and 335.5 nm (log ε 4.41). The second of these maxima is typical for the conjugated system that is formed on the splitting out of OH groups at C-16 and C-14 (see [5]).

The formation of the ester (IV) also showed the presence of a 3β,5β-dihydroxy grouping in S3.

Extremely useful informational material was obtained in a detailed study of the ^1H and ^{13}C NMR spectra of the aglycon S3 and its diacetate in comparison with compounds of known structure — strophanthidin and 17α-strophanthidin (Tables 1 and 2).

In addition to the usual signals in the ^1H NMR spectrum due to the butenolide ring (signals of the 22-H vinyl proton at 6.27 ppm and the 21-methylene group at 4.90 and 5.04 ppm in the form of doublets due to geminal interaction) and to the aldehyde group (singlet at 10.46 ppm) and other signals, we may mention those which have particular value for establishing the structure of the compound under study. Thus, as proof of the presence of a 3β-hydroxy group in S3 we may use the appearance in the weak-field part of the spectrum at 4.41 ppm of the signal of the proton geminal to it. Attention is attracted by the good agreement of the chemical shifts of the protons corresponding to the 3β-hydroxy group, the 19-aldehyde group, and the butenolide ring with the shifts of the corresponding signals in the spectra of strophanthidin and 17α-strophanthidin. From the ^1H NMR spectrum it was also possible to deduce the presence in S3 of one more secondary hydroxy group, to which the signal of the methine protons geminal to it at 4.80 corresponds — obviously 16αH. The above-mentioned conclusions on the structure of cardenolide S3 were also confirmed by the ^{13}C NMR spectrum, which contains the corresponding signals in the expected regions. Moreover, from the ^{13}C NMR spectrum it is possible to draw the conclusion that compound S3 contains tertiary 5β- and 14β-hydroxy groups, to which signals at δ 74.5 and 84.0 ppm, respectively, correspond.

In the ^1H NMR spectra of cardenolide S3 and its diacetate, attention is attracted by the position and form of the signal of the methine proton at C-17, which appears as a doublet with a coupling constant of 8.4 Hz at 3.74 and 3.83 ppm, respectively. From a comparison of its position with the positions of the analogous signals in the spectra of strophanthidin and 17α-strophanthidin it is posible to conclude that the butenolide ring in the molecules of S3 and its diacetate has the α-orientation.

The pronounced downfield shift and the form of this signal permit the assumption that the additional hydroxy group in S3 is located at C-16. We obtained the definitive proof of this by applying the double-resonance procedure to the spectrum of S3 diacetate.

As the result of irradiating a sample with the frequency corresponding to the resonance absorption of the 17β-proton we observed a considerable simplification of the signal at 5.60 ppm of the methine proton geminal to the acetoxy group. In its turn, irradiation with the frequency corresponding to this proton caused the conversion of the doublet 17βH signal into a singlet. This showed unambiguously that the methine proton giving the signal at 5.60 ppm and the acetoxy group geminal to

it were located at C-16 in the molecule of S3 diacetate. The spin-spin coupling constant of the protons at C-16 and C-17 (J = 8.4 Hz) indicates the β-configuration of the 16-hydroxy group.

Thus, the information obtained shows unambiguously that cardenolide S3 has the structure of 16β-hydroxy-17α-strophanthidin (I). In addition, the experimental results given above permit us to represent the conformation of S3 by formula (V). Thus, it has been established that the 16β-OH group has the equatorial position (see the acetylation reaction), and the vicinal protons at C-17 and C-16 occupy axial positions (see the signals in the NMR spectrum at 3.74 and 3.83 ppm with a SSCC of 8.4 Hz).

It is appropriate to mention that 17α-strophadogenin (I) is a native cardenolide of the seeds of *Strophanthus kombe*. We assume that derivatives of it, namely glycosides, should be detected in the plant. In actual fact, when the total native cardenolide glycosides were chromatographed, spots revealed by both the Raymond and the Jensen reagents are observed, in the latter case with the production of a blue fluorescence in UV light.

EXPERIMENTAL

The elementary analyses of the subtances were made with the use of an automatic C–H–N–S analyzer. The results agreed with those calculated for the given structures. Mass spectra were taken on a Varian CH-8 spectrometer (75 eV, 245°C), UV spectra on a Hitachi EP-3 spectrometer, and IR spectra on a UR-10 instrument (KBr tablets).

Optical rotatory dispersion spectra (in methanol) were obtained on a SPU-E automatic spectropolarimeter. ^1H and ^{13}C NMR spectra were recorded in deuteropyridine on a Bruker WM-360 NMR spectrometer with working frequencies of 360 and 90.8 MHz, respectively. Chemical shifts are given relative to TMS, which was used as internal standard. In recording the ^{13}C NMR spectra, for the deuterium stabilization employed in the instrument we used heavy water, D_2O, placed in an external ampul. The solution of the substance under investigation was present in an inserted internal ampul.

The biological activity of S3 was determined on cats using a solution with a dilution of 1:5000. In doses of 0.77 and 0.90 mg/kg there were no signs of near toxicity — the cats behaved calmly.

17α-Strophadogenin. The substance was crystallized from butan-1-ol and twice from ethanol. The crystals were large short prisms. mp 288-289°C, $[\alpha]_D^{20}$ + 65.2 ± 3° (c 0.6 chloroform–methanol–pyridine). Water of crystallization was retained tenaciously. Even after the prolonged drying of the aglycon, elementary analysis showed the composition $C_{23}H_{32}O_7 \cdot 1/2H_2O$. With concentrated sulfuric acid it formed colors changing with time: 0 min — orange; 1 min — red-orange; 5 min — red, remaining unchanged for a long time. This color reaction with sulfuric acid was similar to the analogous reaction for strophadogenin [4, 7].

Acetylation of S3. A solution of 100 mg of the aglycon S3 in 1.5 ml of absolute pyridine was treated with 1 ml of acetic anhydride, and the mixture was left at 20±2°C for 30 h.

By analyzing the course of the reaction with the aid of paper chromatography it was established that the initial cardenolide reacted very rapidly — in the course of 20 min (acetylation of an equatorial OH group). The resulting intermediate monoacetate was then slowly converted into the final product — the diacetate; 2 h 40 min after the beginning of the reaction the monoacetate still remained the predominating cardenolide in the reaction mixture (acetylation of an axial hydroxyl), and acetylation was complete after about 20 h.

After the end of the reaction, the product was precipitated from the reaction mixture with petroleum ether. The precipitate was washed with petroleum ether and was crystallized from a mixture of methanol and water. The yield of crystalline diacetate with mp 212-215.5°C was 75 mg.

The Cyclosulfate (IV). A solution of 30 mg of S3 in 1 ml of anhydrous pyridine was cooled to −10°C, and a solution of 0.04 ml of freshly distilled thionyl chloride in 0.5 ml of benzene was added. In view of the crystallization of the benzene in the reaction mixture (on cooling) the rest of the reaction was carried out at 0°C for 1 h and then at room temperature for 2 h, after which 10 g of ice was added to the reaction mixture. The cardenolides were extracted first with chloroform (4 × 20 ml) and then with chloroform—alcohol (2 × 20 ml).

After the usual washing with water, the combined chloroform and alcohol–chloroform extracts were evaporated in vacuum. The resulting mixtue of cardenolids was chromatographed on 5 g of silica gel. Cardenolide (IV) was eluted with benzene–alcohol (100:0.5) and was crystallized from methanol containing about 10% of water. The substance (IV) obtained had mp 182-184°C.

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