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bis-CARDENOLIDES

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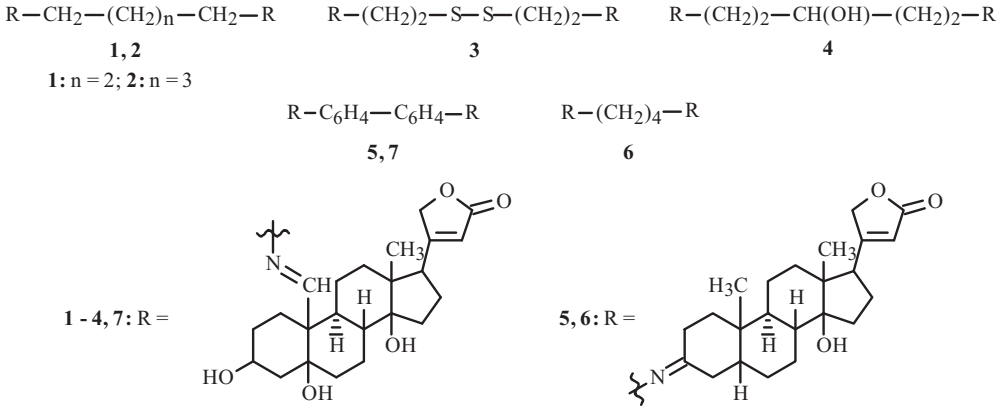
Twelve new bis-*cardenolides were synthesized by reaction of natural cardenolides with diamines to form imines and ammonium salts. Anomalous physicochemical properties consisting of resistance to hydrolysis and increased polarity were observed for the* bis-*ketoimines.*

Keywords: strophanthidine, strophanthidinic acid, strophanthidine-19-carboxylic acid, digitoxigenone, strophanthidine imines, digitoxigenone imines, strophanthidinic acid ammonium salts.

We described in previous reports the preparation of a large array of new aldimines and ketoimines of cardenolides [1–3]. Taking into account the developed methodical approaches for synthesizing these compounds, it seemed possible to prepare a new group of compounds, *bis*-cardenolides, which have not been reported. We used primary diamines $H_2N(CH_2)_nNH_2$, where n = 3 and higher; $H_2N–CH_2–S–S–CH_2–NH_2$, and $H_2N–(C_6H_4)–(C_6H_4)–NH_2$.

In addition, mixed primary, secondary, and tertiary diamines were used to prepare ammonium salts of strophanthidinic acid. The cardenolides strophanthidine, digitoxigenone, and strophanthidinic acid were used in the reaction.

Diimines **1**-**6** were synthesized mainly as before [1]. Certain important points were as follows. The ratio of reactants was strictly controlled in the reaction. Two moles of cardenolide were used per mole of diamine. The hydrocarbon chain of the diamines should contain at least three C atoms. Ketodiimines **5** and **6** were difficult to produce. As a result, the yield of these compounds was very low. The ketocardenolide practically did not react with benzidine at 80°C, the temperature at which all other imines were synthesized. Compound **5** could be obtained in low yield by carrying out the reaction in boiling pyridine, i.e., at a higher temperature (115.3°C). This reaction was also complicated by the presence of a large amount of polymeric products due to decomposition of benzidine and the reaction products.



bis-Ketoimines **5** and **6** were both present as two isomers that were clearly distinguishable on chromatograms. Pure isomers of **6** were obtained using column chromatography over Al_2O_3. Elemental analyses that agreed well with the calculated values and the lack of free amines in the reaction products (according to IR spectra and a negative reaction with ninhydrin) proved that *bis*-ketoimines **5** and **6** had in fact been produced. The IR spectrum of the less polar isomer of **6** (**6b**) exhibited two absorption bands at 1640 and 1660 cm^{-1} that belonged to C=N bonds. This indicated that the C=N bonds in the isomers of **6** were nonequivalent (see below).

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The structure of *bis*-imine **7** was also confirmed using the additivity of the electronic spectra of the cardenolide and the conjugated fragment, diphenyldiamine, at the isobestic point at 212 nm. According to the calculations, the absorption of equimolar solutions in MeOH of the cardenolide and the reaction product should be 1d for the cardenolide, 2d for the condensation product, and 3d for the *bis*-cardenolide. This was confirmed with a high degree of accuracy in the experiment. Solution spectra of the compounds at the calculated concentration intersected at a single point.

The anomalous physicochemical properties of *bis*-ketoimines **5** and **6** were interesting. Unexpectedly for us they turned out to be highly polar compared with starting digitoxigenone. Isomer (a) (more polar) had *R*s of digitoxigenone = 0.013 on TLC using $CHCl_3$:MeOH:H_2O (85:15:0.7); isomer (b), *R*s of digitoxigenone = 0.10. Second, both compounds (**5** and **6**) were difficult to hydrolyze in acidic medium. This was completely uncommon for imines. It is well known that ordinary imines are hydrolyzed simply by water, especially with heating. We think that the reason for these anomalies is as follows.

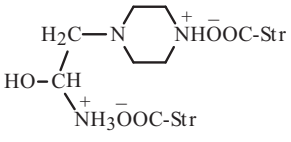
The chemical structures of **5** and **6** are such that their whole non-polar parts are located inside the molecule. They are essentially shielded from the environment. Also, the polar parts of the molecules, i.e., the two lactone rings and two OH groups (on C-14), reside on the outer parts of the molecules and are readily available to all solvents, especially highly polar solvents such as water and alcohols, which form rather strong intermolecular H-bonds. In other words, the hydrophilicity of the molecules and, therefore, their polarities, increase sharply. The difficulty with acid hydrolysis of **5** and **6** is explained by the same molecular structure. The reaction centers at which hydrolysis occurs are C=N bonds, which turn out to be inside the molecule. Access to them of the hydrolyzing agent (H_3O^+) is highly hindered (therefore, they are hydrolyzed with great difficulty).

Returning to the isomerism, let us attempt to determine the structures of the two obtained isomers, primarily for **6**. More polar and less polar isomers are formed in a ratio of about 2:1. It can be concluded from this that the more polar isomer has a more stable stereochemistry. It was theoretically possible that this synthesis could produce three isomers constructed according to the pattern *trans—trans*, *trans—cis*, and *cis—cis*. However, the last structure is improbable. The steric hindrances to its production are insurmountable. Nevertheless, the chromatographic analysis suggests that a small quantity of a third isomer is observed in the reaction mixture (traces). Thus, the two first structures should actually be examined. The isomers can be represented by formulas **6a** and **6b**.

The mutual placement of the N–C bond and the steroidal part of the molecule relative to the C=N bond form the *trans*-(*E*)-isomer (**6a**) or the *cis*-(*Z*)-isomer (**6b**). The non-polar steroidal part in the S part of **6b** is slightly opened and is more accessible to the environment than that in **6a**, which also explains the lower polarity of this isomer. Therefore, we assume that the more polar isomer has structure **6a**; the less polar, **6b**.

The C=N groups in **5b** are nonequivalent. This is confirmed by the IR spectrum (see above).

bis-Salts **7–12** were synthesized by direct reaction of the corresponding diamines and strophanthidinic acid in the strict ratio of 1 mol diamine per 2 moles strophanthidinic acid. The Experimental section gives the reaction conditions.



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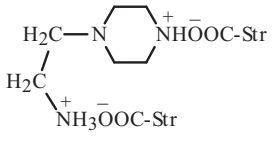

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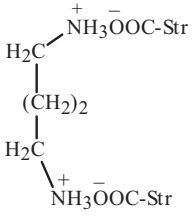

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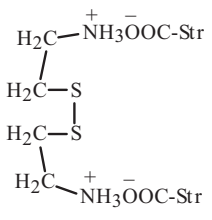

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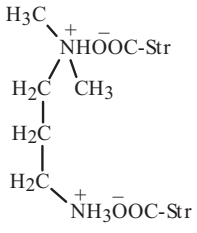

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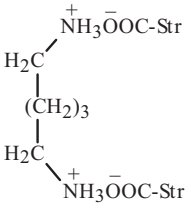

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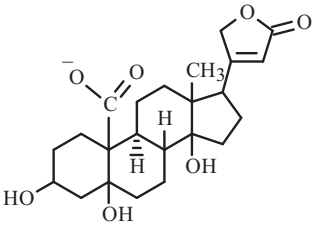
$^-$OOC-Str is the anion of strophanthidinic acid:



EXPERIMENTAL

The course of reactions and purity of products were monitored using TLC on Silufol UV-254 plates. The eluent was $CHCl_3$:MeOH:H_2O (85:15:0.7). Detection used Raymond's reagent. Elemental analyses were carried out on an automated Model 1106 C-H-N-S analyzer and agreed with those calculated for all compounds. IR spectra were recorded on a Specord-75 IR (KBr pellets).

***n*-Pentane-1,5-*N*,*N*-bisaldiminestrophanthidine (2).** Thoroughly dried strophanthidine (2.000 g) was placed into a long-necked flask, dissolved in C_6H_6:*i*-PrOH (2:1), treated with propane-1,5-diamine (0.252 g), and refluxed with periodic addition of solvent. After TLC showed that the reaction mixture contained <50% strophanthidine relative to the product, the solution was concentrated to a syrup and heated further, slowly adding C_6H_6 using a dropping funnel. The reaction was complete after 40 min. Free strophanthidine had almost completely disappeared. The flask was connected to a vacuum to remove remaining solvents. The resulting powdery product was dissolved in *i*-PrOH (10 mL) and stored at room temperature for 40 h. The resulting crystals were separated to afford **2** (1.29 g), $C_{51}H_{74}N_2O_{10}$, mp 232–234°C, $[\alpha]_D^{20}$ +53.9 ± 3° (*c* 1.2, MeOH).

***bis*-Imines 1, 3, and 4** were synthesized analogously: **1**, $C_{50}H_{72}N_2O_{10}$, mp 195–199°C, $[\alpha]_D^{20}$ +49.8 ± 3° (*c* 0.8, MeOH); **3**, $C_{50}H_{72}N_2S_2O_{10}$, mp 178–181°C, $[\alpha]_D^{20}$ +51.2 ± 3° (*c* 0.7, MeOH); **4**, $C_{49}H_{70}N_2O_{11}$, mp 167–170°C, $[\alpha]_D^{20}$ +54.7 ± 3° (*c* 0.9, MeOH).

Digitoxigenone *n*-Butane-1,4-*N*,*N*-bisketoimine (6). Digitoxigenone (1.000 g) and 1,4-diaminobutane (0.118 g) were placed in a flask, dissolved in C_6H_6-*i*-PrOH (4:1, 15 mL), and refluxed, gradually concentrating it to a volume of about 1.5 mL. Benzene was added using a dropping funnel, keeping the volume to 1–1.5 mL. The reaction took 5 h, after which the remaining solvent was removed in vacuo. The solid was chromatographed over a column of Al_2O_3 activated at 115–118°C (2 h). The eluent was $CHCl_3$:MeOH of increasing polarity to a MeOH content of 40%.

Fractions containing two polar cardenolides were evaporated separately. The compounds were crystallized from MeOH:Et_2O.

***bis*-Cardenolide (a)** (more polar, **6a**), $C_{50}H_{72}N_2O_6$, mp >365°C, $[\alpha]_D^{20}$ +24.7 ± 4° (*c* 0.31, MeOH).

***bis*-Cardenolide (b)** (less polar, **6b**), the same formula $C_{50}H_{72}N_2O_6$, mp 120–123/270–272°C, $[\alpha]_D^{20}$ +8.2 ± 4° (*c* 0.42, MeOH). Both compounds gave a negative reaction with ninhydrin (for free amine).

Digitoxigenone Diphenyl-4,4′-*N*,*N*-bisketoimine (5). Digitoxigenone (1.150 g) and benzidine (H_2N–C_6H_4–C_6H_4–NH_2, 0.284 g) were dissolved in pyridine (15 mL); refluxed for 4 h on a glycerine bath, replacing evaporating pyridine from a dropping funnel; and concentrated to a volume of about 5 mL, adding MeOH (30 mL). The resulting resinous precipitate was separated. The solution was evaporated in vacuo. The solid was chromatographed over a column of Al_2O_3 (Brockmann activity level III, 40 g). The eluent was $CHCl_3$:MeOH of increasing polarity. Fractions (5 mL) were collected using an automated collector. Fractions containing the most polar cardenolide were combined and evaporated. The product was crystallized from MeOH:Et_2O to afford **5**, $C_{58}H_{72}N_2O_6$, mp 257–260°C, $[\alpha]_D^{20}$ +21.5 ± 4° (*c* 0.35, MeOH).

***bis*-Strophanthidinebenzidineazomethine (7).** Strophanthidine (1.000 g) and benzidine (0.228 g) were dissolved in anhydrous pyridine (25 mL), placed in a distillation apparatus equipped with a dropping funnel and $CaCl_2$ trap, and heated on a glycerine bath so that the distillation proceeded at 2–10 mL/h, adding constantly at the same rate from a dropping funnel anhydrous pyridine. The reaction took 8 h, after which the bath temperature was lowered to 50°C and pyridine was vacuum distilled to dryness. The solid was chromatographed over a column of Al_2O_3 (Brockmann activity level III, 40 g). The eluent was $CHCl_3$:MeOH of increasing polarity. Fractions (5 mL) were collected using an automated collector. Fractions containing the more polar cardenolide were combined and evaporated. The product was crystallized from MeOH to afford **7**, $C_{58}H_{72}N_2O_{10}$, mp 223–226/247–249°C, $[\alpha]_D^{20}$ +8.4 ± 4° (*c* 0.56, $CHCl_3$:MeOH).

Strophanthidinic Acid *n*-Pentane-1,5-*N*,*N*-*bis*-ammonium Salt (8). Strophanthidinic acid (1.000 g) and *n*-pentane-1,5-diamine (0.120 g) were dissolved separately in MeOH (20 mL each). The solutions were mixed and concentrated in vacuo to a volume of about 3 mL, treated with Et_2O (5 mL), and left at room temperature for 3 h. The resulting crystals were separated and dried to afford **7** (1.05 g), $C_{51}H_{78}N_2O_4$, mp 180–184/215–218°C, $[\alpha]_D^{20}$ +22.6 ± 2° (*c* 1.0, MeOH).

***bis*-Salts 8–12** were prepared analogously: **8**, $C_{52}H_{78}N_3O_{14}$, mp 175–177/201–205°C, $[\alpha]_D^{20}$ +19.2 ± 2°; **9**, $C_{49}H_{74}N_2O_{15}$, mp 205–208°C, $[\alpha]_D^{20}$ +37.0 ± 2°; **10**, $C_{50}H_{76}N_2O_{14}$, mp 188–190/215–220°C, $[\alpha]_D^{20}$ +23.3 ± 2°; **11**, $C_{50}H_{76}N_2S_2O_{14}$, mp 172–175°C, $[\alpha]_D^{20}$ +29.6 ± 2°; **12**, $C_{51}H_{78}N_2O_{14}$, mp 167–170°C, $[\alpha]_D^{20}$ +46.2 ± 2°.

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2. I. F. Makarevich, Yu. I. Gubin, and R. Megges, *Khim. Prir. Soedin.*, 268 (2005).
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