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CARDIOTONIC STEROIDS WITH ADDITIONAL LACTONE RINGS

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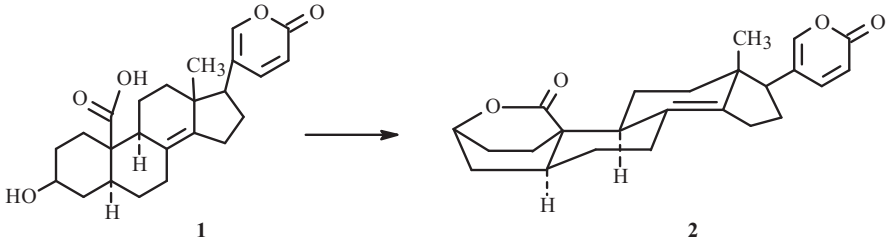
Stereochemical structural transformations of cardiosteroids to create in them additional lactone rings were studied. Creation of δ*-lactones by reaction of a 3*β*-OH group and a 19-carboxylic group to give the corresponding A/B-trans cardiosteroids and creation of* β*-lactones by reaction of a 5*β*-OH group and a 19-COOH to give the corresponding A/B-cis steroids were studied. The new biologically active compounds 8(14)-dehydrobovogenin-19-carboxylic acid 3*β*-O-19-lactone, strophanthidine-19-carboxylic acid 5*β*-O-19-lactone, cymarin-19-carboxylic acid 5*β*-O-19-lactone, convallatoxin-19-carboxylic acid 5*β*-O-19-lactone, and strophalloside-19-carboxylic acid 5*β*-O-19-lactone were prepared.*

Keywords: cardiotonic steroids (cardiosteroids), *β*-lactones, *δ*-lactones, 19-carboxylic acids of cardiosteroids, dehydration, lactonization, hydrolysis.

Ring A in A/B-*trans* steroids becomes fluxional and can convert from the chair to the boat conformation. This causes the 3*β*-OH group and the 19-C functional group to approach each other. We used this feature to react the 19-COOH and the 3*β*-OH to form a *δ*-lactone. The starting compound for this experiment was 8(14)-anhydrobovogenin-19-carboxylic acid (**1**), which was prepared via hydrolysis of bovoside-A-19-carboxylic acid. The reaction was carried out under rather forcing conditions (using HCl, see Experimental) because of the difficulty of hydrolyzing *α*-L-tevetosides such as bovoside A and bovoside-A-19-carboxylic acid. This also eliminated the 14*β*-OH group in the aglycon.



The lactone ring closed to form bufadienolide **2** upon formation of compound **1** by the hydrolysis.

Column chromatography produced pure **2**, the properties of which were investigated. The IR spectrum of **2** showed clearly two lactone rings. The bufadienolide doubly unsaturated six-membered lactone ring was characterized by a strong absorption band at 1738 cm^{-1} (C=O stretching) and two bands at 1633 and 1534 (conjugated C=C stretching). The new 3*β*-*O*-19-lactone gave a strong absorption band at 1715 (C=O stretching). The isolated C=C bond of **2**, being ditertiary, did not appear in the IR spectrum. However, its present was demonstrated by a positive Tortelli–Jaffe reaction [1–3] and by elemental analysis, which corresponded to the formula $C_{24}H_{28}O_4$. As expected, a ditertiary C=C bond formed in the 8:14 position and not a tertiary-secondary bond in the 14:15 position upon elimination of the 14*β*-OH group using HCl [1, 2].

The mass spectrum of **2** gave a strong peak for the molecular ion with *m*/*z* (I_{rel}, 100%) 381.4 $[M + H]^+$.

Formation of the new six-membered 3*β*-*O*-19-lactone and the lack in it of an OH group were confirmed as follows. The IR spectrum did not have the characteristic absorption bands. Furthermore, **2** was not acetylated (by acetic anhydride in pyridine). The disappearance of the 3*β*-OH group indicated unambiguously that it had cyclized with the carboxylic group. As a whole, the results agreed with the proposed structure **2**.

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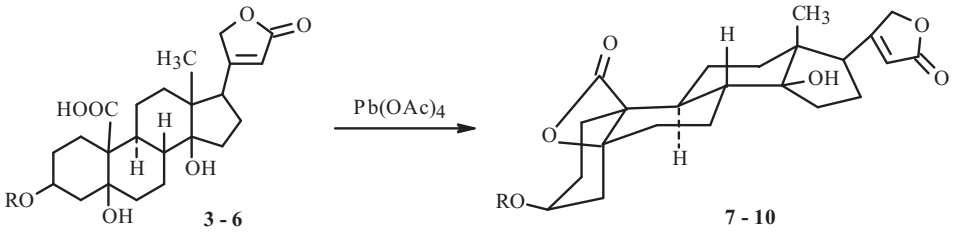


3 - 6 **7 - 10**

3, 7: R = H; **4, 8:** R = β-D-cymarose
5, 9: R = α-L-rhamnose; **6, 10:** R = β-D-allomethylose

The existence of a β-hydroxycarboxylic acid moiety with the *cis* configuration (**3**) among cardiac glycosides and aglycons suggested that lactonization of this group to produce β-lactones was possible. Such a β-hydroxycarboxylic acid moiety occurs for the C_5–C_{10}–C_{19} chain in strophanthidine-19-carboxylic acid (**3**) and its glycosides. The requisite group can be created by oxidation of the angular aldehyde in several cardiac glycosides such as derivatives of strophanthidine, strophadogenin, nigrescigenin, hellebrigenin, antiarigenin, etc. [3].

Whereas δ-lactone **2** formed readily, spontaneously from the corresponding hydroxycarboxylic acids, production of the β-lactones containing a strained four-membered ring required the use of strong dehydrating agents, of which we selected two, acetic anhydride and lead tetraacetate.

Although lead tetraacetate acts as a decarboxylating agent [4], use of it for the β-lactonization reaction turned out to be rather successful. Storing strophanthidine-19-carboxylic acid (**3**) and lead tetraacetate in an anhydrous organic solvent for several hours produced a mixture of cardenolides that was separated by column chromatography. The most polar product turned out to be **7**, called the β-lactone of strophanthidinic acid.

The IR spectrum of the β-lactone showed absorption bands at 1715 and 1799 cm^{-1} (β-lactone C=O stretching), 1736 (butenolide ring C=O stretching), 1625 (butenolide ring C=C stretching), 3429 (broad, OH). One of the last (3β-OH) was due to an intramolecular H-bond with the O atom on C-5.

The PMR spectrum of **7** was consistent with the lack of a carboxylic group and was characterized by a 1H singlet at 5.85 ppm for vinyl proton H-22, a 2H triplet at 4.93 for methylene C-21, a 1H multiplet at 3.95 for proton H-3 geminal to the 3β-OH group [3], and a 3H resonance at 0.72 for the angular 18-methyl group.

The elemental analysis gave the formula $C_{23}H_{30}O_6$.

The mass spectrum (Experimental) showed that the four-membered lactone ring of **7** was exceedingly sensitive to electron impact. The β-lactone lost immediately CO and CO_2 groups. This process was also accompanied by cleavage of ring A and loss of a C_1–C_4 fragment ($C_4H_8O^+ + H^+$) with *m*/*z* 73 (87%). In general, the molecular fragments and their mass numbers agreed with the formula $C_{23}H_{30}O_6$ and the structure **7**.

The β-lactone ring of **7** was hydrolyzed in weakly alkaline solution (Experimental) and the C_3-OH was acetylated in order to prove the structure. Acidification of the reaction mixture, purification over Al_2O_3, and crystallization produced pure strophanthidine-19-carboxylic acid (**3**).

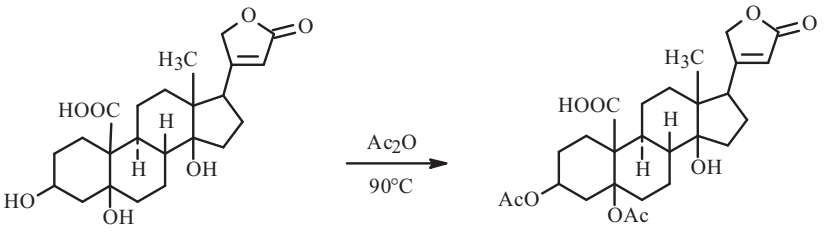
The presence of the 3β-OH group in **7** was also confirmed by acetylation (acetic anhydride in pyridine). The half-reaction time was 3.25 h. This is characteristic of secondary axial OH groups.

In summary, the experimental results indicated unambiguously that the β-lactone with structure **7** was in fact produced.

Stability of the β-Lactone Ring. Crystalline β-lactone **7** could be stored in the cold for long periods without visible changes. Even the crystalline product slowly but noticeably at room temperature formed an impurity of strophanthidine-19-carboxylic acid (**3**), i.e., the β-lactone ring gradually opened.

The analogous β-lactonization occurred for other glycosides **4–6** with strophanthidine-19-carboxylic acid (**3**) aglycon. These glycosides were prepared from cymarin, convallatoxin, and strophalloside via oxidation of the angular aldehyde in them by the method we described earlier [4]. The β-lactonization products **8–10** were isolated pure by column chromatography. Their structures were found by identifying the products of acid hydrolysis. As expected, the common aglycon in them was **7** whereas the carbohydrate components were D-cymarose, L-rhamnose, and D-allomethylose for glycosides **8–10**, respectively.

Use of acetic anhydride to prepare the β-lactones was not as successful. Thus, storing **3** in acetic anhydride at room temperature for 24 h caused no reaction. Heating the solution (90°C, 3 h) produced a mixture of compounds, the principal one of which was 3β,5β-di-*O*-acetylstrophanthidine-19-carboxylic acid (**11**, a new compound).



3 **11**

EXPERIMENTAL

The course of reactions and purity of products were monitored using TLC on Silufol plates. The solvent was $CHCl_3$:MeOH (9:1). Paper chromatography was performed using MEK:*m*-Xyl (1:1)/formamide with detection by Raymond reagent for cardenolides and Lieberman–Burchard for bufadienolides [3, 5]. Elemental analyses were carried out on a model 1106 automated C-H-N-S analyzer and agreed with those calculated.

PMR spectra were taken in DMSO-d_6 on a Mercury VX-200 spectrometer (200 MHz) (Varian) with Me_4Si internal standard; mass spectra, in a 1200L spectrometer (Varian); IR spectra, on a Tensor 27 Fourier-transform spectrometer (Bruker).

8(14)-Anhydrobovogenin-19-carboxylic Acid 3*β*-*O*-19-Lactone (2). Bovoside-19-carboxylic acid (0.5 g) was dissolved in $CHCl_3$:*i*-PrOH (2:1, 120 mL), treated with conc. HCl (1.5 mL), and refluxed for 40 h. The solution was rinsed of HCl by salt solution (15% NaCl, 5 × 5 mL). The neutral solution was evaporated. The solid (0.45 g) was chromatographed over a column of silica gel (0.04–0.06 mm, 40 g) with elution by CCl_4 and CCl_4:$CHCl_3$ (9:1 to 1:9). Fractions of 3–4 mL were collected. Fractions 60–62 were evaporated and crystallized from MeOH to afford **2**, $C_{24}H_{28}O_4$, mp 148–151/179–181°C, $[\alpha]_D^{22}$ +7.2 ± 3° (*c* 0.35, $CHCl_3$).

Strophanthidine-19-carboxylic Acid 5*β*-*O*-19-Lactone (7). Strophanthidine-19-carboxylic acid (**3**, 3 g) was dissolved in $CHCl_3$:*i*-PrOH (1:4, 15 mL), treated with $Pb(OAc)_4$ (4.2 g), stirred on a magnetic stirrer for 5 h, treated with additional $Pb(OAc)_4$ (2 g), and stirred for another 17 h. The resulting small amount of resinous black solid was separated. The solution was diluted with $CHCl_3$:*i*-PrOH (2:1, 70 mL), cooled with ice, and treated with H_2SO_4 (2%) until stably acidic. A copious amount of white lead sulfate precipitated mainly in the upper aqueous phase. The lower organic phase was separated. The upper phase was extracted again with $CHCl_3$:*i*-PrOH (2:1, 3 × 15 mL). The combined organic extracts were washed with water (4 × 10 mL) and evaporated in vacuo.

The product was chromatographed over a column of silica gel (120 g, 0.04–0.06 mm) with elution by CH_2Cl_2 and CH_2Cl_2:MeOH (from 1.5 to 3% MeOH). Fractions of 7–8 mL were collected. Fractions 39–49 produced **7**, $C_{23}H_{30}O_6$, mp 131–133°C (crystallization from MeOH:Et_2O), $[\alpha]_D^{20}$ +17.8 ± 2° (*c* 1.3, MeOH).

The mass spectrum of **7** was characterized by mass numbers (EI, 70 eV, *m*/*z*, I_{rel}, %): 373 (0.3), 372 (10.8), 357 (0.4), 356 (14.4), 336 (0.5), 275 (0.5), 245 (0.7), 219 (24.5), 203 (12.9), 201 (32.4), 193 (15.1), 187 (17.7), 185 (28.6), 183 (22.1), 179 (48.9), 173 (35.8), 165 (44.6), 160 (46.2), 149 (50.8), 143 (59.7), 133 (97.4), 130 (41.2), 128 (90.4), 123 (74.3), 110 (74.5), 106 (73.9), 98 (96.9), 91 (65.4), 83 (36.5), 79 (65.6), 73 (86.9), 70 (93.4), 67 (60.5).

Cymarin-19-carboxylic Acid *β*-Lactone (8). Cymarin-19-carboxylic acid (**4**, 0.5 g) was dissolved in anhydrous DMF (3 mL), treated with $Pb(OAc)_4$ (3 g), held at room temperature for 28 h, diluted with $CHCl_3$:*i*-PrOH (2:1, 50 mL), and treated with H_2SO_4 (2%) until the pH was about 4.5. The lower phase was separated. The upper phase was extracted again with $CHCl_3$:*i*-PrOH (2:1, 3 × 20 mL). The combined organic extracts were washed with water (3 × 15 mL), dried over anhydrous Na_2SO_4, and evaporated. The solid (0.42 g) was chromatographed over a column of silica gel (40 g, 0.04–0.06 mm) with elution by CH_2Cl_2 and CH_2Cl_2:MeOH (from 0.5 to 5% MeOH). Fractions of 3–4 mL were collected. Fractions 16–21 were evaporated and crystallized from Et_2O to afford **8**, $C_{30}H_{42}O_9$, mp 135–137°C, $[\alpha]_D^{20}$ +23.3 ± 3° (*c* 0.33, $CHCl_3$).

Convallatoxin-19-carboxylic Acid *β*-Lactone (9). Convallatoxin-19-carboxylic acid (**5**, 0.4 g) underwent *β*-lactonization as above (for **8**). Chromatography over a column of silica gel isolated **9**, $C_{29}H_{40}O_{10}$, mp 125–127°C (crystallization from MeOH:Et_2O), $[\alpha]_D^{21}$ –9.3 ± 3° (*c* 0.27, MeOH).

Strophalloside-19-carboxylic Acid β-Lactone (10). Strophalloside-19-carboxylic acid (**6**, 0.43 g) underwent β-lactonization as above (for **8**) to afford **10**, $C_{29}H_{40}O_{10}$, mp 138–141°C (crystallization from MeOH:Et_2O), $[\alpha]_D^{22}$ –5.3 ± 3° (*c* 0.25, MeOH).

Hydrolysis of 8. Glycoside **8** (50 mg) was placed into a glass ampul and dissolved in EtOH (1 mL) and H_2SO_4 (0.1%, 1 mL). The ampul was sealed and heated at 80–83°C for 30 min. The aglycon part was extracted from the hydrolysate by $CHCl_3$ (10 mL). The $CHCl_3$ extract was washed with water (3 × 1 mL) and evaporated. The aglycon was crystallized from MeOH:Et_2O, mp 131–133°C. Direct comparison of the aglycon with **7** indicated that they were identical.

Hydrolysis of 9 and 10 was carried out according to Mannich. The compounds (10–15 mg each) were dissolved in Me_2CO:conc. HCl (99:1) and left at room temperature for 30 h. The hydrolysis products were analyzed using TLC. The aglycon in both instances was identified as strophantidinic acid 5β-*O*-lactone (**7**).

Hydrolysis of the β-Lactone Ring. Strophantidinic acid β-lactone (**7**, 50 mg) was dissolved in MeOH (2 mL) and treated with aqueous ammonia (three drops, 25%). TLC monitoring showed that **7** had completely reacted after 3 h. The diluted solution of acetic acid in MeOH (1:9) was added until the pH was about 5.0. The solution was filtered through a layer of silica gel (0.5 g) and evaporated in vacuo. The hydrolysis product was crystallized from *i*-PrOH to afford strophantidine-19-carboxylic acid (**3**, 30 mg), mp 187–190°C, $[\alpha]_D^{20}$ +57.0 ± 3° (*c* 0.5, MeOH).

REFERENCES

1. W. Schlegel, C. Tamm, and T. Reichstein, *Helv. Chim. Acta*, **38**, 1013 (1955).
2. C. Tamm, *Fortschr. Chem. Org. Naturst.*, **13**, 134 (1956).
3. I. F. Makarevich, N. V. Kovganko, I. S. Chekman, and G. V. Zagorii, *Cardiotonic Steroids* [in Russian], Original, Kharkov, 2009.
4. I. F. Makarevich, A. V. Ulesov, and I. A. Nestertsova, *Khim. Prir. Soedin.*, 549 (2009).
5. L. F. Fieser and M. Fieser, *Steroids*, Reinhold Publ. Corp., New York, 1959.
6. I. F. Makarevich, *Khim. Prir. Soedin.*, 221 (1968).