10-CHLORO-19-NORCARDIOSTEROIDS

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Substitution of the angular aldehyde by halogen in cardiosteroids in two steps was studied. The first step was oxidation of the aldehyde and production of the cooresponding 19-carboxylic acids; the second, decarboxylation of the 19-carboxylic acids using N*-chlorosuccinimide or chloride salts. The 10-chloro-19-norcardiosteroids: 10-chloro-19-norstrophanthidine (**1**), 10-chloro-19-norcymarin (**2**), 10-chloro-19-norconvallatoxin (**3**), 10-chloro-19-norstrophalloside (**4**), and 10-chloro-19-norbovoside A (**5**) were prepared for the first time. The intermediates strophalloside-19-carboxylic acid and bovoside A-19-carboxylic acid were also prepared for the first time and characterized.*

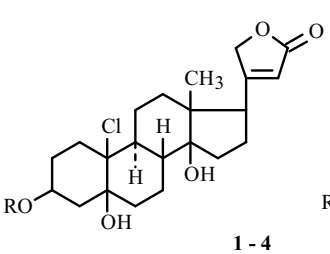
Key words: 10-chloro-19-norcardiosteroids, 19-carboxylic acids of cardiosteroids, angular aldehyde, oxidation, decarboxylation, substitution of carboxylic acid.

Decarboxylation with substitution of the carboxylic acid by halogen is well known in the chemistry of carboxylic acids [1]. It is carried out using lead tetraacetate in the presence of *N*-chlorosuccinimide or metal chlorides. An important feature of this reaction is the lack of structural changes [2].

It seemed interesting to explore the applicability of this method to cardiotonic steroids, namely, to those that contain a tertiary angular 19-COOH group. Positive results would synthesize new biologically active compounds.

19-Carboxylic acids of cardiosteroids are poorly available although they do occur as natural products. Also, they can be prepared rather easily using 19-aldehydes of cardiosteroids, which are available and common in nature [3]. We oxidized the aldehyde group using potassium permanganate, thereby producing in yields up to 85% the corresponding carboxylic acids.

The decarboxylation was monitored by TLC and was carried out in anhydrous DMF, isopropanol, and $CHCl_3$:CH_3OH. Ammonium chloride or *N*-chlorosuccinimide were selected as the chlorides. The reaction was exothermic. Therefore, the formation of side products was reduced by cooling at the start. A researcher carrying out the reaction might get the false impression that the reaction is going excceedingly fast, in 1–2 min. However, TLC analysis shows that the starting acid disappears after 1–2 min and a less polar product appears. If the reaction is stopped even after 15–20 min with such indication by TLC, then the product yield turns out to be very low, about 5% of that calculated. The mistake occurs because the product, taken to be the desired one, is in fact a complex salt formed by the carboxylic acid and the reagent. This complex is an intermediate in the reaction that appears on TLC plates as a real compound and only gradually, over several hours, converts into the desired 10-chloro-19-norcompound, which is less polar than the acid and the complex salt.



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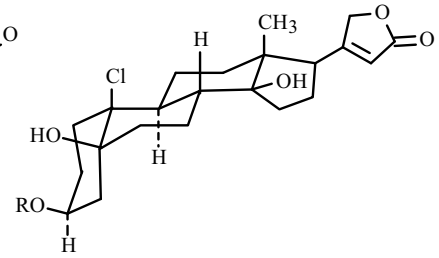

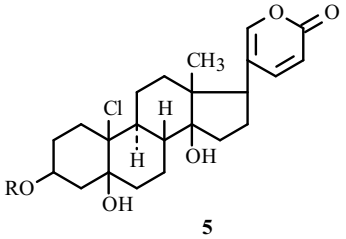

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1: R = H; **2**: R = β-D-cymarose; **3**: R = α-L-rhamnose;
4: R = α-D-allomethylose; **5**: R = α-L-thevetose

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The products were isolated pure, as a rule, using column chromatography over Al_2O_3 or silica gel. The structures of the synthesized compounds (**1**–**5**) were characterized by elemental analysis, a positive Beilstein test (for halogen), IR and PMR spectra, and partly mass spectra.

Thus, the IR spectrum of **1** contained an absorption band at 661 cm^{-1} belonging to the Cl atom (it was absent for the starting 19-carboxylic acid) and bands at 1741 for stretching vibrations of the butenolide ring C=O and 1621 for those of C=C bonds in the ring. A broad band at 3398 cm^{-1} was characteristic of OH groups (3β-OH and 5β-OH) bound in an intramolecular H-bond. The PMR spectrum of **1**, although mediated, clearly showed the Cl substituent on C-10. Vicinal protons on C-1 and C-9 became "visible" under the influence of the elecronegative substituent (Cl-10) and appeared as a 2H resonance at 4.83 ppm and a 1H singlet at 4.20 ppm, respectively. Other resonances included 5.88 ppm, 1H singlet for vinyl proton H-22; 5.33, 2H triplet for methylene C-21; 2.70, 1H multiplet for the C-17 methine; and 0.73, 3H singlet for angular 18-methyl.

Proton H-3, which was geminal to the 3β-hydroxyl, appeared at 3.95 ppm as a 1H multiplet due to coupling with four methylene protons on C-2 and C-4. The coupling constant measured at the very peaks was 3.3 Hz, which was consistent with the equatorial orientation of the proton and agreed with data for strophanthidine [3]. The 18-CH_3 resonance was shifted to strong field due to most probably the through-space influence of the Cl-10 substituent rather than through C–C bonds.

The mass spectrum of **1** was less informative. It did not contain a peak for the molecular ion due to the ease of halogen loss. Facile loss of halogen upon electron impact, especially monosubstituted ones, is a characteristic feature in mass spectrometry [4]. Nevertheless, fragments of the molecule with their mass numbers agreed with the proposed structure of **1**.

Furthermore, the presence of axial 3β-OH in **1** was confirmed by controlled acetylation (acetic anhydride in pyridine). The half-reaction period was 5.5 h, which is typical of secondary axial OH groups [5].

Thus, the results agree unambiguously with the proposed structure of **1** and indicate that decarboxylation of the tertiary angular COOH groups with substitution by a halogen in the cardiosteroids occurs under the described conditions without changing the overall structure of the molecule.

Evidently there is no need to describe in detail the methods for proving the structures of **2**-**4** because the aglycon in them was the same 10-chloro-19-norstrophanthidine (**1**). Acid hydrolysis of them did actually identify the aglycon and the sugar, D-cymarose, L-rhamnose, and D-allomethylose, that corresponded to the natural glycosides used initially in the synthesis.

With respect to the bufadienolide glycoside 10-chloro-19-norbovoside A (**5**), the Cl atom in it was confirmed by the Beilstein test, elemental analysis, the IR spectrum with an absorption band at 647 cm^{-1}, and the PMR spectrum. Like for **1**, the Cl-10 atom, being an electronegative substituent, activated vicinal protons 1CH_2 and 9CH. As a result, they gave a 2H resonance at 4.0 ppm and a 1H resonance at 4.5 ppm.

EXPERIMENTAL

The course of reactions and purity of products were monitored by TLC on Sorbfil plates with elution by $CHCl_3$:CH_3OH (9:1, 85:15) and detection by Raymond reagent for cardenolides and Liebermann–Burckhardt for bufadienolides [3, 6]. Elemental analyses were performed on an automated C-H-N-S analyzer, model 1106, and agreed with those calculated.

PMR spectra were recorded in DMSO-d_6 on a Varian Mercury VX-200 (200 MHz) with TMS internal standard; IR spectra, in KBr disks on a Tensor-27 (Bruker) spectrometer; mass spectra, in a Varian 1200L spectrometer (direct sample introduction into the source, EI 70 eV).

Strophalloside-19-carboxylic acid was prepared by oxidation of strophalloside analogously to that described for erysimin [7]. $C_{29}H_{42}O_{11}$, mp 161–163°C (crystallization from MeOH:Et_2O), $[\alpha]_D^{20}$ +16.1 ± 2° (*c* 1.0, MeOH).

Bovoside A-19-carboxylic acid was prepared by the published method [7]. $C_{31}H_{44}O_{10}$, mp 178–180°C (crystallization from EtOAc), $[\alpha]_D^{20}$ –40.0 ± 2° (*c* 1.0, MeOH).

10-Chloro-19-norstrophanthidine (1). A. Strophanthidine-19-carboxylic acid (1.5 g) was dissolved in anhydrous isopropanol (12 mL) and treated with anhydrous finely ground ammonium chloride (7.5 g) and lead tetraacetate (5.0 g). The lead tetraacetate was added with stirring (magnetic) over 1 h as seven portions. After 3.5 h the mixture was diluted with isopropanol (40 mL). The precipitate was filtered off using a porous filter and vacuum. The filtrate was treated with $CHCl_3$ (100 mL) and saturated aqueous $NaHCO_3$ (40 mL). The resulting white precipitate of lead derivatives was located mainly in the aqueous phase and at the interface. The $CHCl_3$:*i*-PrOH phase was separated, washed with water (15 mL), and evaporated

to dryness. The solid was dissolved with heating in EtOAc (3 mL) and left overnight at room temperature. The resulting crystals were separated and washed with EtOAc to afford **1** (0.45 g), $C_{22}H_{31}O_5Cl$, mp 210–212°C, $[\alpha]_D^{20}$ +17.8 ± 2° (*c* 0.7, MeOH:$CHCl_3$).

B. Strophanthidine-19-carboxylic acid (1.17 g) and *N*-chlorosuccinimide (1.9 g) were dissolved in $CHCl_3$:CH_3OH (5:1, 20 mL), cooled, stirred continuously, and treated with lead tetraacetate (5.37 g). The solution immediately became dark brown. Stirring was continued for 2.5 h at room temperature. Then, the mixture was treated with $CHCl_3$:*i*-PrOH (3:1, 50 mL) and aqueous H_2SO_4 (3%, 20 mL). The lower phase was separated; washed with water (10 mL × 2), saturated $NaHCO_3$ solution (15 mL), and water (10 mL × 2); and evaporated in vacuo. The solid was chromatographed over a column of silica gel (50 g) with elution by CH_2Cl_2:MeOH of increasing polarity. Fractions of 3 mL were collected. Fractions 3–12 produced **1** (0.34 g), mp 210–212°C, $[\alpha]_D^{21}$ +17.4 ± 2° (*c* 0.8, MeOH:$CHCl_3$).

10-Chloro-19-norcymarin (2). The synthesis using cymarin-19-carboxylic acid (1.6 g) was carried out analogously to that for **1** (part **B**) but in anhydrous DMF. The products were chromatographed over a column of Al_2O_3 (Brockmann activity III, 60 g) with elution by CH_2Cl_2 and CH_2Cl_2:MeOH of increasing polarity. Fractions of 3–4 mL were collected. Fractions 24–40 were evaporated. Crystallization of the solid from EtOAc:Et_2O afforded **2** (0.42 g), $C_{29}H_{43}O_8Cl$, mp 144–147°C, $[\alpha]_D^{20}$ +24.1 ± 2° (*c* 0.7, $CHCl_3$).

10-Chloro-19-norconvallatoxin (3). Convallatoxin-19-carboxylic acid (0.5 g) was decarboxylated in the presence of NH_4Cl as described above for **2**. The products were chromatographed over a column of silica gel (30 g, particle size 0.04–0.06 mm) with elution by $CHCl_3$ and $CHCl_3$:MeOH of increasing polarity. Fractions of 2 mL were collected. Fractions 7–12 were evaporated. Crystallization of the solid from MeOH:Et_2O afforded **3** (0.14 g), $C_{28}H_{41}O_9Cl$, mp 128–131°C, $[\alpha]_D^{22}$ –7.3 ± 3° (*c* 0.4, MeOH).

10-Chloro-19-norstrophalloside (4). Strophalloside-19-carboxylic acid (1.4 g) was decarboxylated in the presence of NH_4Cl. The product was purified as described above for **2** to afford **4** (0.37 g), $C_{28}H_{41}O_9Cl$, mp 170–173°C (crystallization from EtOAc:Et_2O), $[\alpha]_D^{20}$ –11.0 ± 2° (*c* 0.7, MeOH).

10-Chloro-19-norbovoside A (5). Bovoside A-19-carboxylic acid (0.8 g) was decarboxylated in the presence of NH_4Cl. The product was purified by the method described for **2** to afford **5**, $C_{30}H_{43}O_8Cl$, mp 121–122°C (crystallization from EtOAc), $[\alpha]_D^{20}$ –33.1 ± 2° (*c* 0.9, MeOH).

Hydrolysis of 2. Compound **2** (10 mg) was placed in a glass ampul and treated with acetic acid (3 drops, 15%). The ampul was sealed and heated at 80–85°C for 10 min. Analysis by TLC and PC showed that the hydrolysis products were 10-chloro-19-norstrophanthidine (**1**) and D-cymarose.

Hydrolysis of 3 and 4 was performed according to Mannich. Samples (10–15 mg) were dissolved in acetone:conc. HCl (99:1), left at room temperature for 30–35 h, and analyzed using TLC. In both instances the aglycon was identified as **1** by direct comparison with an authentic sample.

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