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Colour reaction of cardiotonic cardenolid glycosides with sodium nitroprusside

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Dedicated Prof. Dr. F. Eiden, München, with warm regards on the occasion of his 65th birthday

Digitoxin **(1)** and digitoxigenin **(2)** react with sodium nitroprusside [disodium-pentakis(cyano-C)-nitrosyl-ferrate(II)] under weakly basic conditions forming the corresponding complexes **3** and **4**, which were characterized by elementary analysis and IR-spectra. **3** and **4** are cleaved by cyanide or carbon dioxide/dil. acetic acid to the C-21-oximes **11** and **12**, respectively.

Farbreaktionen von herzaktiven Cardenoliden mit Nitroprussidnatrium

Digitoxin **(1)** und Digitoxigenin **(2)** reagieren mit Nitroprussidnatrium[Dinatrium-pentakis(cyano-C)-nitrosyl-ferrat(II)] unter schwach basischen Bedingungen unter Bildung der entsprechenden Komplexe **3** und **4**, welche durch Elementaranalysen und IR-Spektren charakterisiert wurden. **3** und **4** wurden durch Cyanid bzw. Kohlendioxid/verd. Essigsäure zu den analogen C-21-Oximen **11** und **12** abgebaut.

1. Introduction

There are various colour reactions of cardiotonic glycosides with a cardenolid ring resulting from an interaction of the C–H-acidic O=C–C=C–CH_2–O– increment with electron deficient aromatic compounds under basic conditions, e.g. Baljet reaction (picric acid) and Raymond reaction (m-dinitrobenzene) [1]. According to Kovar [2] Zimmermann-compounds are formed via intermediate Meisenheimer complexes.
Roth et al. [3] have found that C–H-acidic compounds are nitrosylated by disodium-pentakis(cyano-C)-nitrosyl-ferrate(II) (sodium nitroprusside, SNP) under the conditions of the Legal reaction [4]. We have characterized the blue complex formed from acetaldehyde, sec. amines and SNP [5] (Simon-Awe reaction), and Hardegger [6] reports upon a colour reaction of cardenolides with SNP. The analogous cardanolides and isocardenolides, however, do not form coloured complexes [7, 8]. These findings point towards a reaction at the C–H-acidic methylene increment of the unsaturated lactone ring at C-17.

2. Investigations, results and discussion

We have isolated the SNP-complexes of digitoxin (**1**) and of its aglycone digitoxigenin (**2**) and examined them by elementary analysis and degradation reactions.

Concerning the SNP-**1** complex **3** the glycosidicly attached digitoxoses were partially split off under the hydrolytic reaction conditions (cf. Experimental). Therefore, the elementary analysis data for C and H are lower, those for N are higher than the figures calculated for **3**. TLC using digitoxose for comparison shows a weak spot which gets coloured with anisaldehyde/sulfuric acid [9].

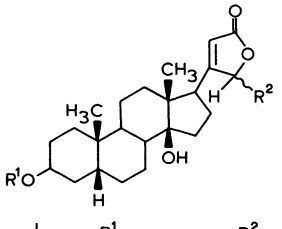
The IR-spectrum of complex **3** shows a weak C=O-absorption (lactone) at 1735–1745 cm^{-1} besides the dominant C≡N-band (5 CN-groups) at 2100 cm^{-1}.

The well known rearrangement of cardenolides to isocardenolides is caused by bases [10, 11]. Therefore, we examined the stability of **1** under our conditions (cf. Experimental): **1** is rearranged in methanol/sodium methoxide slowly, the isocardenolide is detectable by TLC only after 40 min. So this rearrangement can be neglected because our SNP-**1** complex **3** is precipitated after 10 min at the latest.

As stated in the Introduction cardanolides do not react with SNP [7, 8]. In order to correlate this observation with the C–H acidities of the unsaturated/hydrogenated lactones of cardenolides and cardanolides, respectively, we performed H-D exchange experiments [12]. Efforts to hydrogenate **2** to the corresponding cardanolide **12** according to [13–15] (atmospheric pressure, room temperature) failed, addition of acetic acid to the ethanol solution and 10 bar H_2, however, led to 20,22-dihydrodigitoxigenin (**13**).

According to Haberland et al. [16] all the three protons of the unsaturated lactone in cardenolides, e.g. **2**, are exchanged in dimethylformamide using triethylamine as a base, whilst the steroid skeleton remains unaffected: our conditions (CD_3OD/CD_3ONa) yielded the same result. An analogous experiment with the corresponding cardanolid **13** led to incorporation of maximal three D-atoms (FD-MS: d_0: 54%, d_1: 27%, d_2: 15%, d_3: 4%) but this reaction was accompanied by isomerization, as indicated by spectral data [12]. Lindig et al. [17] have reported that the cardanolid **13** is easily hydrolized at the lactone system and that ring closure occurs by acidification with glacial acetic acid. There are, however, no hints given concerning an isomerization.

Digitoxigenin (**2**) when being reacted with SNP in methanol/sodium methoxide formed a violet complex **4**. Elementary analysis established $Na_3C_{28}H_{34}FeN_6O_5$, the IR-spectrum revealed bands at 3400 (OH), 2920 (CH), 2080 (C≡N), 1740 (C=O, lactone) and 1620 cm^{-1} (C=N). These data are in accordance with structure **4**. The stereochemistry at C-21 is unknown because **4** cannot be examined by ^1H NMR-spectroscopy: **4** is either poorly soluble in usual (deuterated) dipolar aprotic solvents or quickly decomposes.

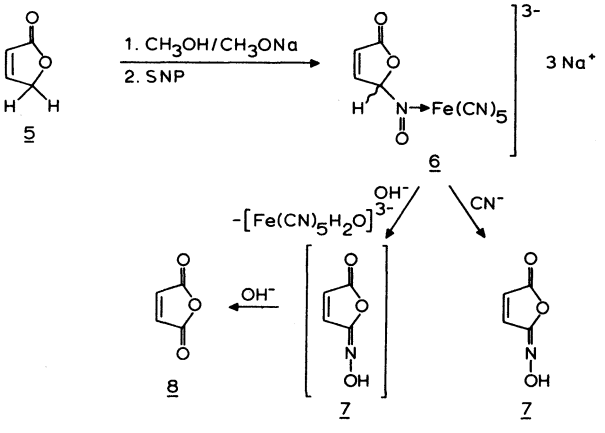


	R^1	R^2
1	$(digitoxose)_3$	H (digitoxin)
2	H	H (digitoxigenin)
3	$(digitoxose)_3$	N→Fe(CN)$_5$, O
4	H	N→Fe(CN)$_5$, O

In order to find suitable cleavage conditions for the SNP-cardenolide complexes we studied the reaction of 2(5 H)-furanone (**5**) with SNP: a violet precipitate **6** arose with correct analytical data for $Na_3C_9H_3FeN_6O_3$ and absorption bands in its IR-spectrum analogous to those of complex **4**.

Cleavage of the SNP-2(5H)-furanone complex **6** with potassium cyanide [5]) led to 5-hydroxyimino-5(H)-furan-2-one (N-hydroxyisomaleimide **7**) showing data identical with those reported by Narita et al. [18] (Scheme 1). Unfortunately we were not able to reproduce Narita's procedure for the preparation of authentic **7**. So, our method seems to be an easy access to this compound. Smooth hydrolysis of complex **6** by 1.5 mol · l^{-1} NaOH according to Roth et al. [3] delivered maleic anhydride (**8**) probably via **7** besides some maleic acid, but no fumaric acid. This hydrolysis converted complex **3** to anhydride **9**, the **2** complex **4** gave the corresponding anhydride **10**.

Scheme 1



Decomposition of **3** and **4**, respectively, with potassium cyanide [5] or cleavage with acetic acid/carbon dioxide according to Küster [19] led to the pertinent nitroso compounds which tautomerize to the oximes **11** and **12** as expected [20]. Oxime **11** is identical with the nitrosation product of **1**, synthesized following the general prescription of Tousters [21] (Scheme 2).

3. Experimental

3.1. Devices

M.p. (uncorr.): apparatus according to Dr. Tottoli (Büchi). IR-spectra in KBr: Beckman Acculab III. ^1H NMR and ^{13}C NMR spectra: Varian EM 390 (90 MHz), Bruker WM 250 (250 MHz), $CDCl_3$, 35 °C, TMS as internal standard. MS: FAB-MS (glycerol, Xe): Varian MAT 311 A.

All reactions were performed under N_2 and light protection between 0 and 5 °C. TLC on SiO_2 (Merck 5554), PTLC on SiO_2, 2 mm (Merck 5717).

3.2. Digitoxin-SNP complex 3

The solution of 765 mg (1 mmol) digitoxin (**1**) and 296 mg (1 mmol) sodium nitroprusside (SNP) in 70 ml of abs. CH_3OH was alkalized by 7 drops of 4 mol · l^{-1} $NaOCH_3$, leading to a deeply red coloured solution. After being acidified with CH_3COOH this solution was evaporated in vacuo to 40 ml and a dark red solid precipitated which was washed several times with ice cold C_2H_5OH until no more **1** was found by TLC. Red precipitate of **3**. IR (cm^{-1}): 3400–3250 (OH); 2100 (C≡N); 1735–45 (C=O, lactone); 1610 (C=N).

$Na_3C_{46}H_{64}FeN_6O_{14}$ (1049.9)

Calcd.: C 52.6 H 6.10 N 8.0
Found: C 50.1 H 5.6 N 9.8

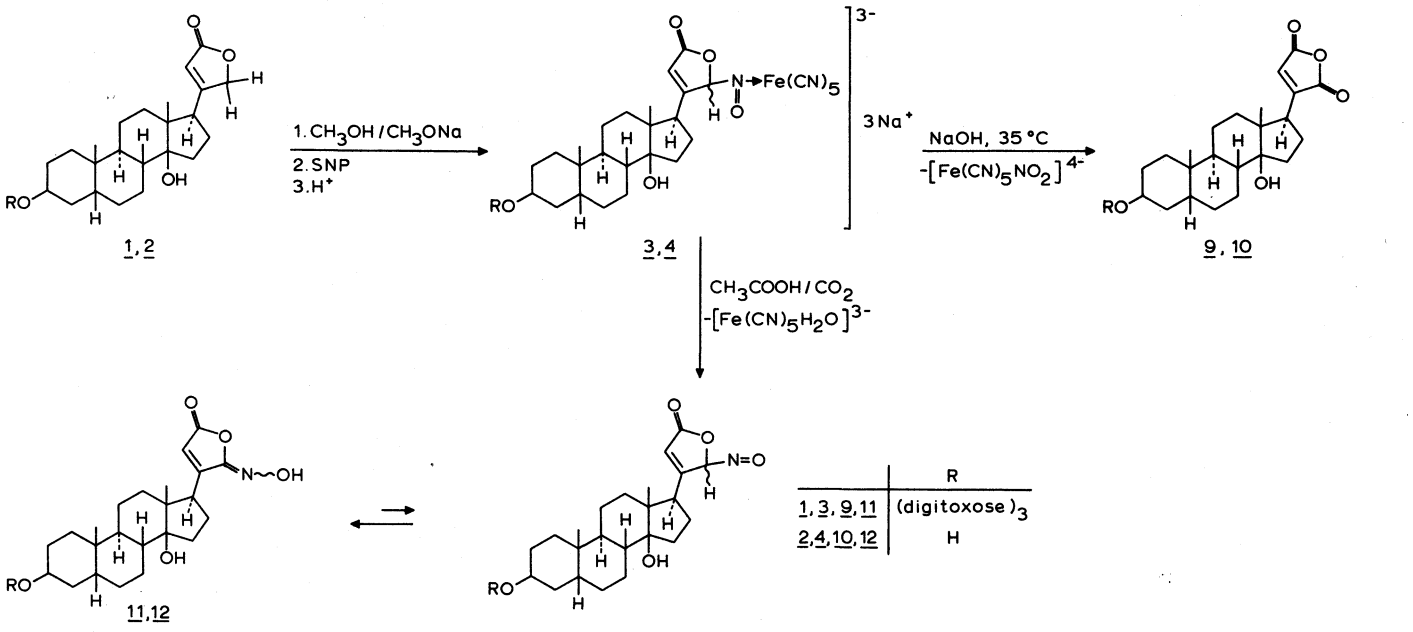
3.3. Digitoxigenin-SNP complex 4

4 is formed from 375 mg (1 mmol) digitoxigenin (**2**) and 296 mg SNP as described for **3** as a red solid. IR (cm^{-1}): 3400 (OH); 2920 (OH); 2080 (C≡N); 1740 (C=O, lactone); 1620 (C=N).

$Na_3C_{28}H_{34}FeN_6O_5$ (659.4)

Calcd.: C 51.0 H 5.16 N 12.7
Found: C 50.6 H 4.94 N 12.6

Scheme 2



3.4. 2(5H)-Furanone-SNP complex **6**

Dropping 0.2 mol of 4 mol · l^{-1} $NaOCH_3$ in CH_3OH to 0.42 g (5 mmol) 2(5H)-furanone (**5**) (Aldrich chemicals) and 1.49 g (5 mmol) SNP in 15 ml of CH_3OH afforded a violet oil which solidified on repeated washing with ice cold C_2H_5OH. Violet solid after drying in vacuo. IR (cm^{-1}): 3400 (OH); 2100 (C≡N); 1745 (C=O, lactone); 1600 (C=N).

$Na_3C_9H_3FeN_6O_3$ (367.9)

Calcd.: C 29.3 H 0.82 N 22.8
Found: C 29.1 H 0.77 N 23.0

3.5. 5-Hydroxyimino-5(H)-furan-2-one (N-hydroxyisomaleimide, **7**)

70 mg of complex **6** are stirred in 10 ml of 0.5 mol · l^{-1} KCN for 3 h. Extraction with CH_2Cl_2, drying (Na_2SO_4) and evaporation in vacuo produced 15 mg (69%) of **7**. M.p. 145 °C (dec.; lit. [14]: 145 °C (dec.)). IR (cm^{-1}): 1770 (CO−O−CN); 1630 (C=N−O).

$C_4H_3NO_3$ (113.07)

Calcd.: C 42.5 H 2.67 N 12.4
Found: C 42.4 H 2.59 N 12.5

3.6. Maleic anhydride (**8**) from complex **6**

100 mg of complex **6**, dissolved in 10 ml aqueous 1.5 mol · l^{-1} NaOH, were kept at 35 °C for 30 min. TLC [diisopropylether/HCOOH/H_2O (90:7:3)] with maleic anhydride, maleic and fumaric acid for comparison shows much maleic anhydride (**8**, R_F 0.5) besides traces of maleic acid (R_F 0.2). Preparative TLC produces 11 mg (41%) maleic anhydride (**8**). M.p. 52–54 °C (authentic sample 54–56 °C). Its IR-spectrum is congruent with that of authentic material.

3.7. 21-Oxo-digitoxin (**9**)

0.2 g of complex **3** were dissolved in 10 ml of aqueous 1.5 mol · l^{-1} NaOH and kept at 35 °C for 30 min. After extraction with $(C_2H_5)_2O$ and usual work-up: 38 mg (26%) **9**. Yellowish crystals. M.p. 189 °C. IR (cm^{-1}): 3250 (OH, br); 1790 (CO−O−CO). NI-FAB-MS: m/z 778 $(M-H)^-$; 650; 520; 390; the pertinent differences correspond to the loss of one molecule of digitoxose (130 mu) each.

$C_{41}H_{62}O_{14}$ (778.9)

Calcd.: C 63.2 H 7.97
Found: C 62.6 H 7.08
Various attempts to get more precise data failed.

3.8. 21-Oxo-digitoxigenin (**10**)

0.2 g of complex **4** were hydrolized and worked up as described for **9**: 37 mg (31%) of **10**. M.p. 137 °C. IR (cm^{-1}): 3300 (OH); 1795 (CO−O−CO). NI-FAB-MS: m/z 387 $(M-H)^-$.

$C_{23}H_{32}O_5$ (388.5)

Calcd.: C 71.1 H 8.25
Found: C 70.9 H 8.17

3.9. 21-Hydroxyiminodigitoxin (**11**)

3.9.1. 0.5 g of complex **3** were dissolved in 10 ml of H_2O and acidified to pH 5 with 2 drops of CH_3COOH. Complex **3** was decomposed by addition of dry ice (solid CO_2). Extraction with $(C_2H_5)_2O$, drying (Na_2SO_4) and evaporation in vacuo produced the oxime **11** as an amorphous solid. M.p. 183 °C (dec.). IR (cm^{-1}): 3300 (OH); 1760 (CO−O); 1620 (C=N−O). ^1H NMR (250 MHz): all the data as known from **1** [22], except δ = 4.92–4.87 ppm (m; 0.4 H, H-21). ^{13}C NMR (250 Hz; δ, ppm): all the data as known from **1** [22] except δ = 157.9 (C-21). PI-FAB-MS: m/z 794 (MH^+), 664 $(MH^+$-digitoxose), 534 $(MH^+$-2 digitoxoses) 404 $(MH^+$-3 digitoxoses), 386 $(MH^+$-3 digitoxoses-H_2O). NI-FAB-MS: m/z 792 $(M-H)^-$, 662 (M-H-digitoxose)$^-$, 531 (M-H-2 digitoxoses)$^-$, 513 (M-H-2 digitoxoses-H_2O)$^-$, 401 (M-H-3 digitoxoses)$^-$, 385 (M-H-3 digitoxoses-H_2O). $[\alpha]_D^{20}$ ($CHCl_3$) = +35°.

3.9.2. 0.1 g of complex **3** were stirred in 10 ml of 0.5 mol · l^{-1} KCN for 3 h. After bleaching the solution became dark blue ("prussian blue"). M.p., elementary analysis and IR-spectrum are identical with those of material obtained in 3.9.1.

3.9.3. To 0.59 g (0.8 mmol) **1** in 50 ml of abs. CH_3OH 0.55 g $NaNO_2$ (8 mmol) in 25 ml CH_3OH were added under stirring at 20 °C. Dropwise addition of fuming HCl afforded a white precipitate (NaCl) which was filtered off by suction after cooling. After evaporated to 1–2 ml, about 15 ml of H_2O were added and **11** was extracted with $(C_2H_5)_2O$. Evaporation and recrystallization from $CHCl_3$ afforded crystals: 104 mg (17%). M.p. 183 °C (dec.).

3.10. 21-Hydroxyiminodigitoxigenin (**12**)

0.1 g complex **4** were treated as described for compound **11** (procedure 3.9.1.) leading to 35 mg (57%) **12**. M.p. 199 °C. IR (cm^{-1}): 1745 (CO−O), 1650 (C=N−O). ^1H NMR (250 MHz; δ, ppm): 5.87 (s; 1 H, H-22), 4.92–4.87 (m; 0.4 H, H-21), the other data as known from lit. [22]. ^{13}C NMR (250 Hz; δ, ppm): all the data known for **2** [22] except δ = 157.9 ppm (C-21). PI-FAB-MS: m/z 404 (MH^+), 389, 307, 263.

$C_{23}H_{33}NO_5$ (403.5)

3.11. 20,22-Dihydrodigitoxigenin (**13**)

100 mg of **2** in 25 ml of abs. C_2H_5OH and 3 drops of glacial CH_3COOH were hydrogenated with 40 mg of Pt-black at 10 bar H_2 and room temperature for 26 h. After filtration and evaporation in vacuo the oily residue was crystallized and recrystallized from $CHCl_3$: white needles, m.p. 224 °C (lit. [13]: 226 °C, CH_3OH). ^1H NMR; δ, ppm): 4.54–4.35 (m, 2 H, H-21), 2.5–1.8 (m, 3 H, H-20 and H-22), all the other data are found as described for **12** except the signals at δ = 5.87 and 4.92–4.87 ppm.

3.12. H/D exchange experiments with digitoxigenin (**2**) and 20,22-dihydrodigitoxigenin (**13**)

To 100 mg of **2** or **13**, respectively, in 1 ml of CD_3OD were added 2 drops of $NaOCD_3$ and CD_3OD (40% solution, from Na^0 and CD_3OD). After 5 min the mixture was slightly acidified by DCl (25% solution), Merck reagents) and evaporated at 40 °C in vacuo. OD groups were reexchanged to OH groups by 3 ml of H_2O, then the organic material was extracted with abs. $(C_2H_5)_2O$. Crystallization from $CDCl_3$ led to d_3-**2**. M.p. 254 °C (m.p. of **2**: 255–256 °C [23]). ^1H NMR (δ, ppm): the singlet for H-22 at 5.87 and the doublet of C-21-CH_2, J = 18 Hz, in **2** are missing.

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