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Reactivity of Pyrrole Pigments, Part XVII [1]. Reduction of Bile Pigments by Sodium Dithionite

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Summary. Bilanediones can be easily obtained by sodium dithionite reduction of the corresponding alkyl-substituted bilindiones or biladiene-*ac*-diones. By reduction of vinyl-substituted dipyrrinones with sodium dithionite, the corresponding alkyl-substituted 4,5-dihydrodipyrrinones can be obtained.

Keywords. Bilirubin; Biliverdin; Urobilinogen.

Reaktivität von Pyrrolpigmenten, 16. Mitt. [1] Reduktion von Bile-Pigmenten mit Natriumdithionit

Zusammenfassung. Bilandione können auf einfache Art aus den entsprechenden alkylsubstituierten Bilin- order Biladien-*ac*-dionen durch Reduktion mit Natriumdithionit hergestellt werden. Durch Reduktion von vinylsubstituierten Dipyrrinonen mit Natriumdithionit erhält man die entsprechenden alkylsubstituierten 4,5-Dihydrodipyrrinone.

Introduction

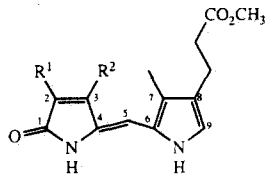
Bilirubin-IXα (**4a**), the end product of heme catabolism in mammals, is formed by biliverdin reductase catalyzed reduction of biliverdin-IXα (**3a**). Subsequently, the lipophilic bilirubin **4a** is transformed into a more polar and easily excretable product by esterification with glucuronic acid. Once in the intestine, the excreted pigment undergoes a series of not well known stepwise reductions, performed by bacterial enzymes, to urobilinogen (**5a**) and stercobilinogen [2].

Urobilinogen has usually been obtained from bilirubin (**4a**), either by Pd catalyzed hydrogenation in NaOH solution [3] or by sodium amalgam reduction [4]. The yields of urobilinogen obtained by catalytic hydrogenation are in the range of 20%–40%, while in the sodium amalgam method the yield is about 60%. However, owing to the alkaline conditions, products with pyrrolidone end rings are also obtained [5]. The nature of the reaction products is also very sensitive to the reaction conditions (basicity of the medium, reaction time, and sodium amalgam concentration), suggesting a sequence of events in the sodium amalgam reduction of **4a** [6].

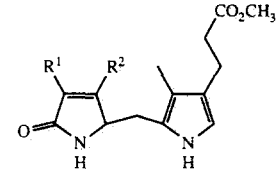
The biladiene-*ac*-diones (*e.g.* bilirubin) can be considered as two dipyrrinones (structure **1**) joined through position 9 by a methylene bridge. Therefore, dipyrrinones have been extensively used as models of biladiene-*ac*-diones [7]. We have already shown that the exocyclic double bond of alkyl-substituted dipyrrinones can be

reduced with sodium dithionite, affording the corresponding 3,4-dihydrodipyrrinone [8]. However, since natural bile pigments have vinyl groups on their lactam rings, the reduction of their model dipyrrinones with a vinyl group either on position 2 (*exo*) or 3 (*endo*) had to be studied too.

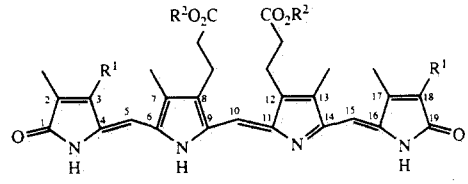
The conjugated system of bilindiones is extended over the four pyrrole rings, the central bridge being the most electron-deficient position. Therefore, a stepwise $Na_2S_2O_4$ reduction of the three exocyclic double bonds (from bilindiones to bilandiones through biladiene-*ac*-diones) should also be possible. In fact, *Fischer* has already described the sodium dithionite reduction of biliverdin-IXα (**3a**) to bilirubin-IXα (**4a**) in aqueous KOH at room temperature in 3% yield [9]. Mesobiliverdin-IXα and mesobiliverdin-IXα dimethyl ester (**3b**) have also been reported to afford mesobilirubin-IXα and mesobilirubin-IXα dimethyl ester (**4c**), respectively, by reduction with sodium dithionite, but reaction conditions and the yields were not specified [10]. We expected, however, that with stronger reduction conditions these reactions would not stop at this stage. Therefore, we studied the $Na_2S_2O_4$ reduction of bilindiones and biladiene-*ac*-diones and vinyl-substituted dipyrrinones.



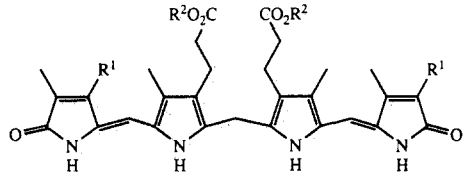
	R^1	R^2
1a	CH_3-	CH_2=CH-
1b	CH_2=CH-	CH_3-



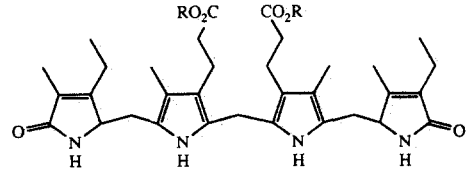
	R^1	R^2
2a	CH_3-	CH_3-CH_2-
2b	CH_3-CH_2-	CH_3-



	R^1	R^2
3a	CH_2=CH-	H-
3b	CH_3-CH_2-	CH_3-



	R^1	R^2
4a	CH_2=CH-	H-
4b	CH_2=CH-	CH_3-
4c	CH_3-CH_2-	CH_3-



	R
5a	H-
5b	CH_3-

Results and Discussion

Optimization of the sodium dithionite reduction conditions for alkylsubstituted dipyrrinones led us to the here so-called 'standard reduction conditions'. These experimental conditions are: large excess of $Na_2S_2O_4$ with respect to the substrate, stepwise addition of the reductant, enough $NaHCO_3$ to maintain the solution slightly basic ($pH \approx 8$), reaction temperature of 90 °C, and minimum of DMF:H_2O (1:1) to get the dipyrrinone dissolved at 90 °C [8].

The reduction of vinyl-substituted dipyrrinones **1a** and **1b** with sodium dithionite, under standard reduction conditions, afforded the alkyl-substituted 4,5-dihydrodipyrrinones **2a** and **2b**. These products correspond to the reduction of both the vinyl and the exocyclic double bond. However, unlike the reduction of alkyl-substituted dipyrrinones, vinyl-substituted dipyrrinones never gave yields of reduced derivatives higher than 50% (Table 1). A large amount of starting material was transformed into polar water soluble species which remained in the aqueous phase after extraction with organic solvents. The formation of polar water-soluble by-products occurs by subsidiary reactions characteristic of some sodium dithionite reductions. These side-reactions can be accounted for by addition of sulfoxylate anion to the vinyl groups [11]. In conclusion, these results of the reduction of vinyl-substituted dipyrrinones with sodium dithionite also explain the difficulties that we have later encountered in the sodium dithionite reduction of other vinyl-substituted bile pigments.

Bilirubin (**4a**) is not soluble under standard reduction conditions. However, a relatively stable supersaturated solution of bilirubin can be obtained by addition of a small amount of NH_4OH to a suspension of bilirubin in DMF:H_2O (1:1), followed by $NaHCO_3$ addition (final $pH \approx 8$). After treatment of this supersaturated solution with sodium dithionite at 90 °C for several hours, only water soluble reduction products (not extractable with organic solvents even at $pH \approx 3.5$) were obtained. The maximum at 213 nm in the UV/Vis spectrum suggests a bilanedione type structure, while the shoulder at 398 nm points to the presence of small amounts of bilenedione type structures.

Table 1. Reaction products and yields of some representative reductions with sodium dithionite in DMF:H_2O (1:1) at 90 °C

Substrate	Reduction product	Reaction time (h)	Yield[a] (%)
1a	**2a**	1	12
1a	**2a**	2	28
1b	**2b**	1	50
3b	**5b**	1.5	80
4c	**5b**	2	54

[a] See experimental part

Bilirubin dimethyl ester (**4b**) does not present solubility problems; therefore, standard reduction conditions could be applied. Nevertheless, a complex mixture amounting to less than one third of the original mass was obtained in the organic phase, urobilinogen dimethyl ester (**5b**) being one of the components. The remaining reaction products could only partially be extracted from the acidified ($pH \approx 3.5$) aqueous phase, affording a complex mixture too. In conclusion, the reduction of the vinyl-substituted biladiene-*ac*-diones **4a** and **4b** with sodium dithionite resembles, at a higher degree of complexity, that found in vinyl-substituted dipyrrinones, and thus has no preparative utility.

Mesobilirubin-IXα dimethyl ester (**4c**) neither contains vinyl groups on the lactam rings nor presents solubility problems. After reduction of **4c** with sodium dithionite under standard conditions, we obtained a mixture containing more than 80% of urobilinogen dimethyl ester (**5b**). HPLC separation afforded the two racemic mixtures of **5b** in a 1:1 proportion and 54% overall yield (Table 1).

Owing to our previous results in the reduction of vinyl-substituted biladiene-*ac*-diones and the low yield described by *Fischer* [9] in the reduction of biliverdin (**3a**), we have not attempted the reduction of vinyl-substituted bilindiones. Instead, we have reduced an alkyl-substituted bilindione, mesobiliverdin dimethyl ester (**3b**), with sodium dithionite under standard conditions. The reaction mixture contained the two racemic mixtures of **5b** in a 1:1 proportion and 80% overall yield (determined by ^{1}H NMR spectroscopy). In fact, no significant difference has been found between the reduction of mesobilirubin dimethyl ester (**4c**) and the reduction of mesobiliverdin dimethyl ester (**3b**).

In conclusion, the sodium dithionite reduction of bilindiones or biladiene-*ac*-diones can be used as a method for the preparation of urobilinogens when the starting material belongs to the *meso* type, *i.e.* in the absence of vinyl substituents. To avoid solubility problems or the use of more basic media, the reduction is better performed *via* the methyl esters instead of the free carboxylic acids.

Experimental

Melting points were determined on a Kofler-Reichert micro hot-stage apparatus. UV/Vis spectra were recorded on a Perkin-Elmer Lambda 5 instrument, IR spectra on a Perkin-Elmer 681 spectrometer, Mass spectra (MS) on a Hewlett-Packard 5988A instrument equipped for FAB analysis with a Capillaritron Frasor and ^{1}H NMR spectra were recorded on a Varian XL-200 spectrometer (200 MHz). High pressure liquid chromatography (HPLC) was carried out on Radial Pak silica or C18 columns with a Waters double pump using a variable wavelength detector 5FA 339; preparative HPLC at the semimicro scale was carried out using a Porasil 15 cm × 19 mm column (Waters).

Bilirubin was obtained from Sigma. Sodium dithionite was obtained from Panreac, and its purity (73%) was determined according to a method described in the literature [12]. All the reduction experiments were carried out at 90 °C under an Ar atmosphere using Ar-saturated solvents and magnetically stirring the mixture. The reaction work-up involved cooling the reaction mixture to room temperature, evaporation to dryness, redissolution in $CHCl_3/H_2O$, extraction of the aqueous layer with $CHCl_3$, drying of the combined organic phases over anhydrous Na_2SO_4, and evaporation to dryness. The preparation and properties of the following compounds are described in the literature: **1a** [13, 14], **1b** [13, 14], **3b** [6], **4b** [15], and **4c** [15, 16].

Preparation of (±)-3-ethyl-8-(2-methoxycarbonylethyl)-2,7-dimethyl-4,5-dihydrodipyrrin-1(10H)-one **2a** *by reduction of (Z)-8-(2-methoxycarbonylethyl)-2,7-dimethyl-3-vinyldipyrrin-1-(10H)-one* **1a**

Experiment A: A total of 218 mg (0.9 mmol) of $Na_2S_2O_4$ and 238 mg (2.8 mmol) of $NaHCO_3$ were divided in two equal portions and added at 0.5 h intervals to a solution of 20 mg (0.066 mmol) of **1a** in 4 ml of *DMF*:H_2O (1:1) (reaction time: 1 h). Following the standard work-up, 3 mg of a crude material were obtained. HPLC and ^1H NMR of this material showed that it contained 80% of **2a** [17]. Calculated yield: 12%.

Experiment B: A total of 205 mg (0.9 mmol) of $Na_2S_2O_4$ and 223 mg (2.7 mmol) of $NaHCO_3$ were divided in three equal portions and added at 0.6 h intervals to a solution of 10 mg (0.033 mmol) of **1a** in 2.5 ml of *DMF*:H_2O (1:1) (reaction time: 2 h). Following the standard work-up, 4 mg of a crude material were obtained. HPLC and ^1H NMR of this material showed that it contained 71% of **2a** [17]. Calculated yield: 28%.

Preparation of (±)-2-ethyl-8-(2-methoxycarbonylethyl)-3,7-dimethyl-4,5-dihydrodipyrrin-1-(10H)-one **2b** *by reduction of (Z)-8-(2-methoxycarbonylethyl)-3,7-dimethyl-2-vinyldipyrrin-1(10H)-one* (**1b**)

A total of 110 mg (0.46 mmol) of $Na_2S_2O_4$ and 118 mg (1.4 mmol) of $NaHCO_3$ were divided in two equal portions and added at 0.5 h intervals to a solution of 10 mg (0.033 mmol) of **1b** in 2 ml of *DMF*:H_2O (1:1) (reaction time: 1 h). Following the standard work-up, 5 mg (0.017 mmol) of **2b** [17] were obtained. Yield: 50%.

Reduction of bilirubin **4a**

To a suspension of 100 mg (0.17 mmol) of **4a** in 40 ml of *DMF*:H_2O (1:1), the minimum amount of NH_4OH was added to get the bilirubin dissolved. Addition of 200 mg (2.4 mmol) of $NaHCO_3$ and 600 mg (5.7 mmol) of Na_2CO_3 brought the solution to $pH \approx 8$. To this supersaturated solution, heated at 90 °C, a total of 823 mg (3.5 mmol) of $Na_2S_2O_4$ divided in 5 equal portions were added at 1.5 h intervals. After 7.5 h, the yellow reaction mixture was evaporated to dryness, redissolved in H_2O and unsuccessfully extracted with different organic solvents (*i.e.* $CHCl_3$, Et_2O, and AcOEt). Acidification of the aqueous phase to $pH \approx 3.5$ didn't allow to extract any product, either.

The UV/Vis spectrum of the aqueous phase in CH_3OH showed a maximum at 213 nm with shoulders at 278 nm and 398 nm.

Reduction of bilirubin dimethyl ester **4b**

A total of 546 mg (2.3 mmol) of $Na_2S_2O_4$ and 594 mg (7.1 mmol) of $NaHCO_3$ were divided in three equal portions and added at 0.5 h intervals to a solution of 50 mg (0.082 mmol) of **4b** in 21 ml of *DMF*:H_2O (1:1) (reaction time: 1.5 h). Following the standard work-up, 15 mg of a mixture were obtained. Acidification of the aqueous phase to $pH \approx 3.5$ allowed to extract an additional amount of 22 mg. TLC (SiO_2, CH_2Cl_2/EtOH 100/5), IR (KBr), FAB-MS and ^1H NMR ($CDCl_3$) analysis showed that urobilinogen-type compounds were the main components of both extracted fractions. Urobilinogen dimethyl ester (**5b**) was one of the components, and no starting material was detected.

Preparation of urobilinogen dimethyl ester **5b** *by reduction of mesobilirubin dimethyl ester* **4c**

A total of 983 mg (4.1 mmol) of $Na_2S_2O_4$ and 1067 mg (13 mmol) of $NaHCO_3$ were divided in three equal portions and added at 0.6 h intervals to a solution of 26 mg (0.042 mmol) of **4c** in 28 ml of

DMF:H_2O (1:1) (reaction time: 2 h). Following the standard work-up, 26 mg of a crude material containing the two racemic mixtures of **5b** were obtained. Semipreparative HPLC separation (Porasil, CH_2Cl_2:EtOH 1000:57, 8 ml·min^{-1}) afforded two main fractions with retention times of 5.9 and 9.2 min. Each fraction contained 7 mg (0.0113 mmol) of a racemic mixture of **5b**. Overall yield: 54%

Less polar racemic mixture of **5b**: TLC (SiO_2, CH_2Cl_2:EtOH 1000:35): $R_f = 0.35$; HPLC (Porasil, CH_2Cl_2:EtOH 1000:57, 8 ml·min^{-1}): retention time 5.9 min; ^{1}H NMR ($CDCl_3$, 200 MHz, δ): 9.31 and 9.25 (2 s, broadened, 2 pyrrolic NH), 7.97 and 7.83 (2 s, broadened, 2 lactamic NH), 4.24 (m, H-4), 4.09 (m, H-16), 3.77 and 3.65 (AB system, $J_{AB} = 16.7$ Hz, H_2C-10), 3.63 and 3.60 (2 s, 2 $COOCH_3$), 3.1–2.1 (m, H_2C-5, H_2C-15, 2 CH_3OOC–CH_2–CH_2-, 2 CH_3–CH_2-), 1.94, 1.86 and 1.79 (3 s, 3 CH_3), 1.67 (s, H_3C-2), 1.12 and 0.85 (2 t, 2 CH_3–CH_2-, $J = 7.5$ Hz); IR (KBr, cm^{-1}): 3310, 1740, 1680; UV/Vis (CH_3OH): 208 nm; MS (*m/z* (%)): 620 (M^+, 1), 496 (77), 371 (100), 317 (9), 180 (44), 124 (22).

More polar racemic mixture of **5b**: TLC (SiO_2, CH_2Cl_2:EtOH 1000:35): $R_f = 0.25$; HPLC (Porasil, CH_2Cl_2:EtOH 1000:57, 8 ml·min^{-1}): retention time 9.2 min; ^{1}H NMR ($CDCl_3$, 200 MHz, δ): 8.37 (s, broadened, 2 pyrrolic NH), 6.27 (s, broadened, 2 lactamic NH), 4.09 (m, H-4), 3.96 (m, H-16), 3.80 and 3.75 (AB system, $J_{AB} = 16.3$ Hz, H_2C-10), 3.64 (s, 2$COOCH_3$), 3.1–2.1 (m, H_2C-5, H_2C-15, 2CH_3OOC–CH_2-CH_2-, 2CH_3–CH_2-), 1.94, 1.93 and 1.90 (3s, 3CH_3), 1.74 (s, H_3C-2), 1.09 (t, CH_3–CH_2-3, $J = 7.3$ Hz), 0.98 (t, CH_3–CH_2-18, $J = 7.3$ Hz); IR (KBr, cm^{-1}): 3330, 1740, 1680; UV/Vis (CH_3OH): 208 nm; MS (*m/z* (%)): 620 (M^+, 1), 496 (72), 371 (100), 317 (13), 180 (45), 124 (31).

Preparation of urobilinogen dimethyl ester **5b** *by reduction of mesobiliverdin dimethyl ester* **3b**

A total of 3.17 g (13 mmol) of $Na_2S_2O_4$ and 3.44 g (41 mmol) of $NaHCO_3$ were divided in two equal portions and added at 0.75 h intervals to a suspension of 19 mg (0.031 mmol) of **3b** in 58 ml of *DMF*:H_2O (1:1) (reaction time: 1.5 h). Following the standard work-up, we obtained 17 mg of a crude material containing the two racemic mixtures of **5b** in a 1:1 proportion and 90% overall yield (determined by ^{1}H NMR). Calculated yield: 80%.

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