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On the Preparation of Bilirubins of the Natural α Series Substituted with a Propionic Acid Residue and a Hydroxypropyl Group [1]

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Summary. The reduction of mesobiliverdin XIIIα propan-1,3-diyl diester with $NaBH_4$ affords mesobilirubin XIIIα propan-1,3-diyl diester. The same reduction of mesobiliverdin XIIIα methylen diester. The same reduction of mesobiliverdin XIIIα methylen diester affords 8-(2-carboxyethyl)-3,17-diethyl-12-(3-hydroxypropyl)-2,7,13,17-tetramethylbiladien-*ac*-1,19-(21*H*, 24*H*)-dione (**MBR-mc**). The UV/Vis and ^{1}H NMR spectra of **MBR-mc** show that its structure in solution is similar to that of the natural bilirubins of the α series.

Keywords. Acylal; Bile pigment; Biliverdin; Cyclic esters; Selective reduction.

Zur Herstellung von Bilirubinen der natürlichen α-Reihe mit Propionsäure- und Hydroxypropylsubstituenten

Zusammenfassung. Reduktion von Mesobiliverdin-XIIIα-propan-1,3-diyl-diester mit $NaBH_4$ liefert Mesobilirubin-XIIIα-propan-1,4-diyl-diester. Die analoge Reaktion des Mesobiliverdin-XIIIα-methylen-diesters führt zu 8-(2-Carboxyäthyl)-3,17-diäthyl-12-(3-hydroxypropyl)-2,7,13,17-tetramethylbiladien-*ac*-1,19-(21*H*,24*H*)-dion (**MBR-mc**). **MBR-mc** weist in Lösung (UV/Vis und 1H-NMR Spektren) eine ähnliche Struktur auf wie die der natürlichen Bilirubine der α-Serie.

Introduction

The reduction of biliverdins to bilirubins is an easy process which is currently accomplished for preparative purposes by using $NaBH_4$ in methanol (*e.g.* [2]). $NaBH_4$ affords the reduction of the biliverdin π system to that of the bilirubin and does not affect the acid or ester groups of the bile pigment substituents.

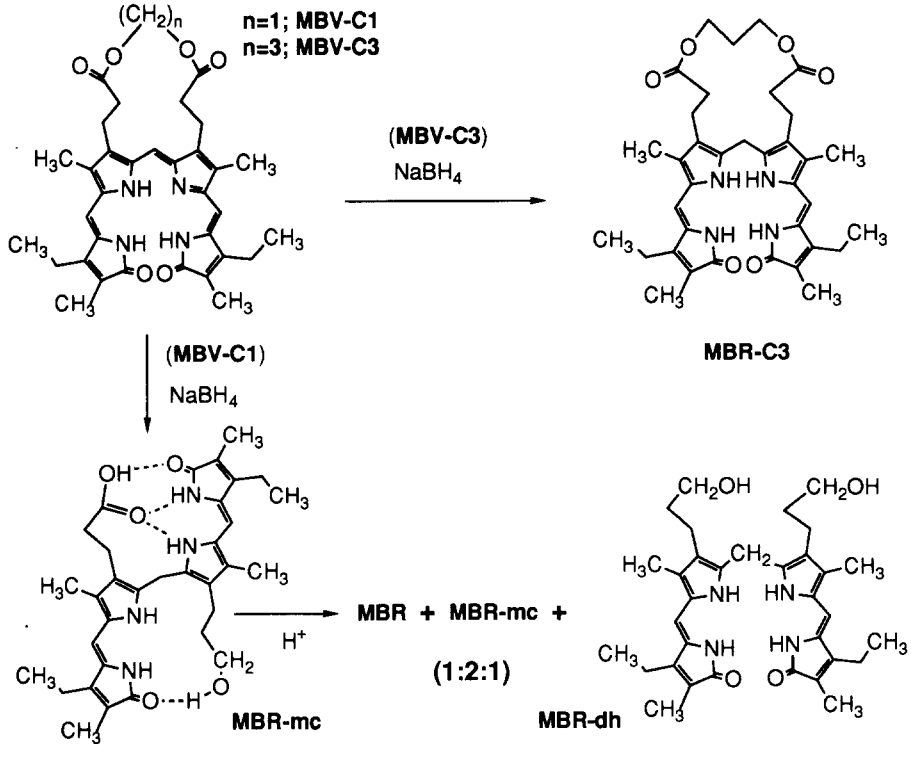
Recently, we have published preliminary results on the $NaBH_4$ reduction of mesobiliverdin IXα methylene ester to the corresponding internaly cyclized bilirubin [3]. New experiments on the symmetrical analog mesobiliverdin XIIIα methylene ester (**MBV-C1**) show that in fact the end product obtained corresponds to a bilirubin with one of the propionic acid substituents reduced to the primary alcohol (**MBR-mc**; see Scheme 1). Such reductions can be performed in methanol or in tetrahydrofuran, the reaction being much slower in the latter. However, in the case of methanol yields decrease because of the partial hydrolysis of the starting material. This selective ester reduction to a half-ester may be attributed to an effect of the

acylal group, because in the case of the propan-1,3-diyl ester of mesobiliverdin XIIIα (**MBV-C3**) the reaction product corresponds to the internal cyclized bilirubin diester (**MBR-C3**).

To our knowledge, there is no reference in the literature referring to this effect of the reduction. The activation of the carbonyls of the acylal diester group to $NaBH_4$ reduction compared to a normal ester group may be attributed to the presence of a second oxygen in the alkyl residue. Furthermore, only one of the two carbonyl groups is reduced, which could be explained by a) a low reactivity to $NaBH_4$ of the first reduction product, and b) a different reactivity of the two carbonyl groups due to different chemical environments originating in the macrocycle conformation. In this sense, simple molecular mechanics calculations [3] show that the two carbonyl groups have clearly different environments due to the conformational equilibrium of the internally cyclized biliverdin. Further work to clarify this reduction mechanism and to evaluate its potential as a general synthetic method is in progress.

Results and Discussion

MBR-mc easily undergoes the so-called scrambling reaction [2a, 5]. This results in the formation, besides with **MBR-mc**, of mesobilirubin XIIIα (**MBR**) and the corresponding dihydroxy mesobilirubin (**MBR-dh**). **MBR-dh** shows a low solubility in $CHCl_3$ and is soluble in methanol. In TLC, **MBR-mc** shows an intermediate polarity between those of **MBR** and **MBR-dh**, and a lower solubility in $CHCl_3$ than **MBR**. In the presence of air, **MBR-mc** shows a stability to oxidation, similar to that



of **MBR**, but **MBR-dh** is easily oxidized to biliverdins typical of the non-propionic acid substituted bilirubins. This suggests that the ridge tile conformation of **MBR** is preserved for **MBR-mc** but not for **MBR-dh**. The ^{1}H NMR and UV/Vis spectra agree with this hypothesis.

The UV/Vis behaviour of **MBR-mc** is similar to that of **MBR**, *i.e.*, it shows a similar absorption ($\approx$420 and 390 (sh) nm; intensity ratio 5:4) which does not change significantly when varying the concentration or the solvent (*DMSO*, $CHCl_3$, and $CHCl_3/CH_3OH$). This corresponds to the ridge tile conformation with intramolecular hydrogen bonds between the carboxylic acid groups and hydrogens of the dipyrrinone halves [2a, 4]. The double absorption band is interpreted to be due to exciton coupling between the two dipyrrinone halves. In contrast, the UV/Vis spectra of **MBR-dh** show a concentration dependence of the intensity ratio of the two exciton coupling bands; changes are also observed upon varying the solvent ($CHCl_3$, $CHCl_3/CH_3OH$, and *DMSO*); these changes are similar to those of bilirubin dimethyl esters.

The ^{1}H NMR spectrum of **MBR-mc** shows two distinct dipyrrinone moieties, both with NOESY effects, corresponding to the (*Z*)-*syn* arrangement of the dipyrrinone halves and to the ridge tile conformation of the bilirubins of the α series with propionic acid substituents [2a, 6]. Two of the four N-H signals show the characteristic chemical shifts originating from the internal hydrogen bonding of the lactamic and pyrrolic NH with one of the propionic acid substituents [7] ($CDCl_3$: 9.25 ppm pyrrolic, 10.75 ppm lactamic; *DMSO*-d_6. 9.85 ppm lactamic, 10.1 ppm pyrrolic). The other two NH signals show the same characteristic change when varying the solvent from $CDCl_3$ to *DMSO*-d_6 but with respect to a typical bilirubin they are shifted towards higher magnetic fields in $CDCl_3$ (pyrrolic: 8.85 ppm, lactamic: 9.30 ppm) and to lower magnetic fields in *DMSO*-d_6 (10.35 ppm lactamic, 10.60 ppm pyrrolic). These results suggest that the association between the primary alcohol and dipyrrinone in **MBR-mc** occurs because of the stability of the predominant hydrodgen bonding mainly between the propionic acid and the dipyrrinone. This accounts for the significant influence of the carboxylic group–NH–NH interaction on the structure of bilirubin.

Experimental

Mesobiliverdin XIIIα methylene ester (**MBV-C1**) and *Mesobiliverdin XIIIα propan-1,3-diyl ester* (**MBV-C3**)

MBV-C1 and **MBV-C3** were obtained by the general method described in Refs. [3, 8] in 45% and 50% yields.

MBV-C1: MS(+)-FAB (Xe, NBA): m/z = 599 (M + 1); UV/Vis (CH_2Cl_2, λ_{max} (nm), ε): 365 (45800), 631 (13000); ^{1}H NMR ($CDCl_3$, 300 MHz, δ (ppm)): 8.20 (broad s, NH), 6.71 (s, HC=, C10), 5.92 (s, HC=, C5 and C15), 5.79 (s, O-CH_2-O), 2.97 (m, C8 and C12-CH_2-CH_2-COO), 2.62 (m, CH_2-CH_2-COO), 2.52 (q, C3 and C17-CH_2-CH_3), 2.09 (s, C7 and C13-CH_3), 1.83 (s, C2 and C18-CH_3), 1.22 (t, C3 and C17-CH_2-CH_3).

MBV-C3: MS(+)-FAB (Xe, NBA): m/z = 627 (M + 1), 649 (M + Na); UV/Vis (CH_2Cl_2, λ_{max} (nm), ε): 366 (50700), 628 (13900); ^{1}H NMR ($CDCl_3$, 300 MHz, δ = (ppm)): 8.30 (braod s, NH), 6.76 (s, HC=, C10),

5.88 (s, HC=, C5 and C15), 4.26 (broad t, 2 × O-CH_2), 2.94 (m, C8 and C12-CH_2-CH_2-COO), 2.54 (m, CH_2-CH_2-COO), 2.50 (q, C3 and C17-CH_2-CH_3), 2.07 (s, C7 and C13-CH_3), 1.95 (m, O-CH_2-CH_2-CH_2-O), 1.82 (s, C2 and C18-CH_3), 1.20 (t, C3 and C17-CH_2-CH_3).

$NaBH_4$ reduction of **MBV-C1** *and* **MBV-C3**

About 20 mg (0.037 mmol) of biliverdin were slowly added to a suspension of 14 mg (0.0037 mmol) of $NaBH_4$ in 15 ml *THF*. The solution was stirred under an Ar atmosphere at room temperature and in the dark just to conversion to bilirubin color (≈90 min). Addition of 100 ml CH_2Cl_2 and washing with water afforded after drying of the organic phase with $NaSO_4$ and evaporation a residue which was purified by PTLC using CH_3OH/CH_2Cl_2 as eluent. From **MBV-C1** or **MBV-C3** the bilirubins **MBR-mc** or **MBR-C3** were obtained in 50% and 89% yields, respectively.

Mesobilirubin XIIIα propan-1,3-diyl ester (**MBR-C3**)

UV/Vis (*DMSO*; λ_{max} (nm), ε): 416 sh, 387 (8:9); ^{1}H NMR ($CDCl_3$, 300 MHz, δ (ppm)): 10.65 (broad s, lactam NH), 10.43 (broad s, pyrrole NH), 5.94 (s, HC=, C5 and C15), 4.36 (m, 2 × O-CH_2), 4.12 (s, CH_2 C10), 2.92 (m, C8 and C12-CH_2-CH_2-COO), 2.51 (m, CH_2-CH_2-COO), 2.35 (q, C3 and C17-CH_2-CH_3), 2.12 (s, C7 and C13-CH_3), 2.09 (m, O-CH_2CH_2-CH_2-O), 1.52 (s, C2 and C18-CH_3), 1.02 (t, C3 and C17-CH_2-CH_3).

3,17-diethyl-8-(2-carboxyethyl)-12-(3-hydroxypropyl)-2,7,13,17-tetramethylbiladien-ac-1,19-(21H,24H)-dione (**MBR-mc**)

MS(+)-FAB (Xe, NBE): m/z = 575 (M + 1), 597 (M + Na); UV/Vis ($CHCl_3$, λ_{max} (nm), ε): 420, 390 sh (5:4); ^{1}H NMR ($CDCl_3$, 300 MHz, δ(ppm)): 10.75 (s, N24-H), 9.30 (s, N21-H), 9.25 (s, N23-H), 8.85 (s, N22-H), 6.07 and 5.92 (s, C5 and C15 HC=), 4.00 (broad s, C10 CH_2), 3.84 (broad s, CH_2-OH), 2.84 (m, CH_2-CH_2-COOH), 2.77 (m, CH_2-CH_2-COOH and CH_2-CH_2-CH_2OH), 2.49 and 2.47 (q, C3 and C17-CH_2-CH_3), 2.17 (s, C7 and C13-CH_3), 1.87 and 1.84 (s, C2 and C18-CH_3), 1.83 (m, CH_2-CH_2-CH_2OH), 1.13 and 1.12 (t, C3 and C17-CH_2-CH_3).

Scrambling reaction of **MBR-mc**

Treatment of a solution of **MBR-mc** with traces of acid results in the formation of mesobilirubin XIIIα, **MBR-mc**, and **MBR-dh** in a ratio of 1:2:1. **MBR-dh** was isolated by PTLC.

3,17-diethyl-8,12-bis(3-hydroxypropyl)-2,7,13,17-tetramethylbiladien-ac-1,19-(21H,24H)-dione (**MBR-dh**)

UV/Vis (*DMSO*, λ_{max} (nm), ε: 423, 396 sh (11:10); ^{1}H NMR ($CDCl_3/CH_3OD$ (10:1), 300 MHz, δ (ppm)): 6.05 (s, HC=, C5 and C15), 3.96 (s, CH_2, C10), 3.71 (t, 2 × CH_2-OH), 2.71 (t, 2 × -CH_2-CH_2-CH_2OH),2.50 (q, C3 and C17-CH_2-CH_3), 2.11 (s, C7 and C13-CH_3), 1.87 (s, C2 and C18-CH_3), 1.85 (m, 2 × CH_2-CH_2-CH_2OH) 1.14 (t, C3 and C17-CH_2-CH_3).

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