Monatshefte für Chemie 120, 575—580 (1989)

Monatshefte für Chemie
Chemical Monthly

On the Fate of Biliverdin-IIIα-dimethyl Ester Formed by Scrambling During Syntheses of Biliverdin-IXα-dimethyl Ester from Bilirubin

Daniel Krois and **Harald Lehner***

Institut für Organische Chemie, Universität Wien, A-1090 Wien, Austria

Summary. In preparations of biliverdin-IXα-dimethyl ester (**2b**) from bilirubin-IXα (**1**) the ratio of the XIIIα- and IIIα-isomers **3b** and **4b**, formed via intermolecular scrambling, should be unity. However, irrespective of the synthetic variant considered, the amount of **4b** obtained usually is exceptionally low. This is partly ascribed to a consecutive reaction of **4b** in acidic methanol affording the chiral diastereomeric bridged biliverdins **5**(**a** und **b**) and **6**(**a** and **b**), respectively.

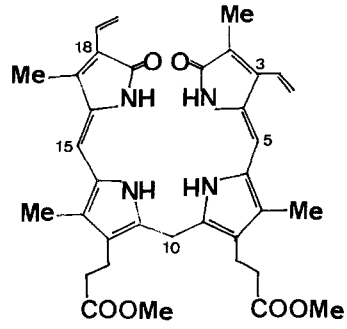
Keywords. Biliverdin-IIIα; Bridged biliverdins; Bilatrienes.

Zum Verbleib des durch Scrambling gebildeten Biliverdin-IIIα-dimethylethers bei Synthesen des IXα-Isomeren aus Bilirubin

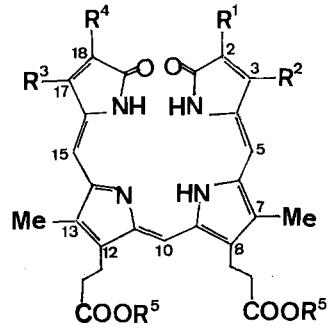
Zusammenfassung. Bei der Darstellung von Biliverdin-IXα-dimethylester (**2b**) aus Bilirubin-IXα (**1**) sollte das Verhältnis der durch intermolekulares Scrambling gebildeten XIIIα- und IIIα-Isomeren **3b** bzw. **4b** eins betragen. Tatsächlich aber ist die Ausbeute an **4b**, unabhängig von der verwendeten synthetischen Variante, immer sehr klein. Dieser Umstand läßt sich zumindest teilweise auf eine spezifische Reaktion von **4b** in saurem Methanol zurückführen, in deren Verlauf die chiralen diastereoisomeren überbrückten Biliverdine **5**(**a** und **b**) sowie **6**(**a** und **b**) gebildet werden.

Introduction

Biliverdins are model compounds for the prosthetic group of naturally occurring biliproteins. The function of these chromoproteins is diverse and has intensely been reviewed in recent years [1]. Conveniently biliverdin-IXα-dimethyl ester (**2b**) is used because it makes allowance for further derivatisation and may easily be prepared from commercially available bilirubin-IXα (**1**). Two main synthetic methods have been applied: a one-step and a two-step synthesis [2–5]. In the former **1** is treated with acidic methanol containing an appropriate oxidans, mostly ferric trichloride, while in the latter procedure oxidation and esterification are performed successively. Due to reversible acid catalyzed cleavage about the C-10 methylene bond of **1** [2] both routes occasionally afford appreciable amounts of the XIIIα-isomer ester **3b** and as a minor component the IIIα-isomer **4b**. By contrast, the bilatriene system once formed does not suffer further isomerization. If the one step synthesis is considered, large amounts of methoxybiliverdins are formed on account of **2b** and **4b** because of the high reactivity of vinyl groups

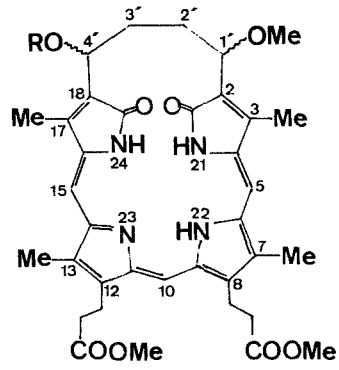


1



	R^1	R^2	R^3	R^4	R^5
2a	Me	$CH=CH_2$	Me	$CH=CH_2$	H
2b	Me	$CH=CH_2$	Me	$CH=CH_2$	Me
3a	Me	$CH=CH_2$	$CH=CH_2$	Me	H
3b	Me	$CH=CH_2$	$CH=CH_2$	Me	Me
4a	$CH=CH_2$	Me	Me	$CH=CH_2$	H
4b	$CH=CH_2$	Me	Me	$CH=CH_2$	Me

located at the C-2 and C-18 position of the biladien system towards electrophilic addition [3]. This reaction occurs prior to oxidation [6] and effects the yield of **2b** but especially that of **4b** while it has no influence on the amount of **3b** formed. However, even if the two step synthesis is used, thus avoiding the side reactions mentioned above, the yield of **4b** generally remains low [4, 7, 8] even if by scrambling equal amounts of **3b** and **4b** are to be expected. Strikingly, if more vigorous esterification conditions, e.g. methanol sulfuric acid, are employed only traces of **4b** are obtained [8]. This must be ascribed to a general susceptibility of **4b** towards acidic conditions. However, no attempt has been made hitherto to put forward a more precise chemical interpretation for this peculiarity. In the course of our attempts into the synthesis of biliverdins with fixed cyclic geometry we recently have found



5 R = H

6 R = Me

a [(P,1'R,4'R) + (M,1'S,4'S)]

b [(M,1'R,4'R) + (P,1'S,4'S)]

a specific reaction of **4b** towards acidic methanol affording chiral diastereoisomeric bridged compounds [9]. This reaction was suspected to be responsible for the low yield of **4b** in the two step synthesis.

We therefore undertook a more careful investigation into the products formed during the most efficient biliverdin dimethyl ester preparation procedure, i.e. oxidation of **1** with 2,3-dichloro-5,6-dicyanobenzochinon (*DDQ*) followed by methylation with methanol – sulfuric acid. We report in this paper some of our results.

Results and Discussion

The biliverdin ester preparation presented in the experimental section afforded a crude product which was chromatographed on silica gel. Elution was started with dichloromethane – acetone (30 : 1 *v/v*) to give the isomeric biliverdin esters **2b**, **3b**, and **4b**. The amount of **2b** is approximately twice as large as that of **3b** but sixty times larger than that of the IIIα-isomer **4b**.

The following product distribution (mg) was obtained from a biliverdin preparation through oxidation of 1.5 g of **1** with *DDQ* followed by esterification with acidic methanol:

2b	**3b**	**4b**	**5**	**6**
450	210	15	8	55

The bridged, more polar diastereoisomeric compounds **6** (**a** and **b**) and **5** (**a** and **b**) were obtained in that order by gradient elution with dichloromethane – acetone increasing the proportions from 30 : 1 to 30 : 6 *v/v* [10]. Isomers **6a** and **6b**, and **5a** and **5b** are thermally interconvertible due to *M/P* helix-inversion which (though retarded by the four membered bridge) proceeds with sufficient rate at ambient temperatures [11, 12].

On the basis of complete randomization the isomer ratio **2b**:**3b**:**4b** should be 2:1:1. This requirement approximately fits for **2b** and **3b** but apparently fails if **4b** is considered. Clearly the disappearance of **4b** in biliverdin preparations is equivalent to the appearance of **5** and **6**. The reaction pathway includes intramolecular cyclization, oxidation, and reaction with methanol and water [9]. One might argue that formation of the bridged compounds **5** and **6** proceeds via intramolecular oxidative coupling of vinyl groups of the bilirubin system simultaneously with its oxidation to a bilatriene and subsequent addition of methanol or water to the double bonds of the intermediate bridged diene thus generated. However, if bilirubin-IIIα is oxidized with *DDQ* [13] no diene can be detected and the product mainly consists from **4a**. Moreover, if the products formed by oxidation of **1** with *DDQ* are analyzed prior to esterification the isomer ratio **2a**:**3a**:**4a** amounts to 2:1.1:0.9 and approximately fits statistical expectations. The elucidation of isomer distribution was performed by ^{1}H nmr, drawing a comparison with the individual spectra of **2a**, **3a**, and **4a**. These results are in accord and corroborate other findings on the pathway of this reaction. As may be concluded from Ref. [9] both the rate of formation and the yield of the bridged compounds **5** and **6** depend on the initial concentration of **4b**, acidity, and the amount of water. In addition, even if the major isomer **2b** present in solution cannot undergo cyclization it likewise promotes the reaction serving as an oxidizing agent like **4b** itself. This oxidation step is presumed to be rate limiting and the participation of **2b** has been demonstrated [9]. From the consideration above can be concluded that a modification of the product distribution may occur if the conditions for methylation are even slightly changed.

In conclusion, even if other side reactions may additionally account for the low yield of **4b** in biliverdin preparations the consecutive appearance of **5** and **6** may at least be regarded as important contribution [14]. Moreover, in all reactions in which biliverdin-IIIα-dimethyl ester (**4b**) or another bilatriene possessing olefinic groups at the C-2 and C-18 positions is subjected to acidic conditions one must be aware of the additional formation of bridged compounds.

Experimental

M.p.s. were determined with a Kofler-Reichert hot stage apparatus. ^{1}H nmr spectra (250 MHz) were measured with a Bruker WM 250 instrument using tetramethylsilane as an internal standard. UV-visible absorption spectra were obtained with a Perkin Elmer Lambda 7 instrument. All reactions were performed under protection from light. Solvents used were deaerated by three freeze-pump-thaw cycles and flushed with argon prior to use. The isomeric purity of the bilirubin-IXα (**1**) (Serva) used was checked by ^{1}H nmr to be >95%. *DDQ* was purchased from Merck.

Oxidation of Bilirubin-IXα (**1**) *with DDQ Followed by Esterification with Acidic Methanol: Biliverdin-IXα-dimethyl ester* (**2b**), *Biliverdin-XIIIα-dimethyl ester* (**3b**), *Biliverdin-IIIα-dimethyl ester* (**4b**), *[(P,1'R,4'R)+(M,1'S,4'S)]- and [(M,1'R,4'R)+(P,1'S,4'S)]-2,18-(1'-Methoxy-4'-hydroxybutane-1',4'-diyl)-8,12-bis(2''-methoxycarbonylethyl)-3,7,13,17-tetramethyl-1,19-(21H,24H)-bilindion* (**5a**) *and* (**5b**), *and [(P,1'R,4'R)+(M,1'S,4'S)]- and [(M,1'R,4'R)+(P,1'S,4'S)]-2,18-(1',4'-Dimethoxybutane-1',4'-diyl)-8,12-bis(2''-methoxycarbonylethyl)-3,7,13,17-tetramethyl-1,19-(21H,24H)-bilindione* (**6a**) *and* (**6b**)

1 (1.5 g, 2.57 mmol) was dissolved in *DMSO* (180 ml) and a solution of *DDQ* (1.35 g, 5.95 mmol) in *DMSO* (75 ml) was added under vigorous stirring within 5 min. After 1 min at 20°C the mixture was

poured into water (500 ml) containing ascorbic acid (2 g) and acetic acid (0.5 ml). After centrifugation the supernatant was discarded and the amorphous precipitate washed twice with 0.001 *N* aqueous acetic acid. After drying 1.5 g of a dark powder was obtained which was mainly a mixture of **2a**, **3a**, and **4a** (2 : 1.1 : 0.9) (nmr). This material was dissolved in methanol – sulfuric acid (20 : 1 *v/v*) (40 ml) and stirred at room temperature for 24 h. The dark green solution was then filtered and neutralized with saturated aqueous sodium hydrogen carbonate. After stripping with chloroform the extract was washed three times with water, dried (sodium sulfate), and the solvent evaporated *in vacuo*. Column chromatography (Kieselgel 60, 230–400 mesh, Merck; dichloromethane – acetone applying a gradient from 30 : 1 up to 30 : 6 *v/v*) afforded compounds **4b**, **2b**, **3b**, **6** (**a** and **b**), and **5** (**a** and **b**) in that order. They were identical with authentic samples obtained from different sources: **2b** [7], **3b** [7], **4b** [4, 9], **5** [9], **6** [9], with respect to m.p., t.l.c., ^{1}H nmr, and uv-visible spectra.

Biliverdin-IXα (**2a**)

Prepared from **2b** by saponification according to Ref. [15]; ^{1}H nmr (*DMSO-d*$_6$, δ): 10.26 (1 H, br s, H-21 or H-24), 10.03 (1 H, br s, H-21 or H-24), 7.02 (1 H, s, H-10), 6.82, and 5.72 (3 H, AXY, J_{AX} 17.8 Hz, J_{AY} 11.5 Hz, 3-vinyl group), 6.56, 6.07, and 5.38 (3 H, AMX, J_{AM} 17.5 Hz, J_{AX} 11.5 Hz, J_{MX} 2.5 Hz, 18-vinyl group), 6.14 (1 H, s, H-5 or H-15), 6.11 (1 H, s, H-5 or H-15), 2.85 (4 H, m, CH_2-8, CH_2-12), 2.42 (4 H, m, CH_2COO), 2.17 (3 H, s, CH_3-17), 2.08 (3 H, s, CH_3-7 or CH_3-13), 2.06 (3 H, s, CH_3-7 or CH_3-13), and 1.80 (3 H, s, CH_3-2).

Biliverdin-XIIIα (**3a**)

Prepared from **3b** by saponification according to Ref. [15]; ^{1}H nmr (*DMSO-d*$_6$, δ): 10.45 (2 H, br s, H-21, H-24), 7.22 (1 H, s, H-10), 6.82 and 5.70 (6 H, AXY, J_{AX} ca. 18 Hz, J_{AY} ca. 12 Hz, 3- and 17-vinyl group), 6.09 (2 H, s, H-5, H-15), 2.82 (4 H, m, CH_2-8, CH_2-12), 2.28 (4 H, m, CH_2COO), 2.03 (6 H, s, CH_3-7, CH_3-13), and 1.85 (6 H, s, CH_3-2, CH_3-18).

Biliverdin-IIIα (**4a**)

Bilirubin-IIIα (18 mg, 0.03 mmol) [16] was dissolved in *DMSO* (2.25 ml). Within 5 min a solution of *DDQ* (16.2 mg, 0.07 mmol) in *DMSO* (0.9 ml) was added and the mixture kept for 5 min at room temperature. The dark green solution was then poured into water (30 ml) containing ascorbic acid (0.1 g) and centrifugated. The supernatant was discarded and the precipitate washed twice with water. This afforded after drying 17 mg **4a** (95%): ^{1}H nmr (*DMSO-d*$_6$, δ): 10.14 (2 H, br s, H-21, H-24), 7.08 (1 H, s, H-10), 6.54, 6.01, and 5.39 (6 H, AMX, J_{AM} 16 Hz, J_{AX} 10.8 Hz, $J_{MX} \approx 2$ Hz, 2- and 18-vinyl group), 6.13 (2 H, s, H-5, H-15), 2.86 (4 H, m, CH_2-8, CH_2-12), 2.43 (4 H, m, CH_2COO), 2.17 (6 H, s, CH_3-3, CH_3-17), and 2.08 (CH_3-7, CH_3-13).

Acknowledgements

This work has been supported by the Hochschuljubiläumsstiftung der Stadt Wien and by the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich (projects 4009 and P5767).

References and Notes

[1] For reviews see: Scheer H. (1981) Angew. Chem. **93**: 230; Angew. Chem. Int. Ed. Engl. **20**: 241; Ó Carra P., Ó hEocha C. (1976) In: Goodwin T. W. (ed.) Chemistry and Biochemistry of Plant Pigments, Vol. 1, 2nd edn. Academic Press, New York, p. 328

[2] McDonagh A. F. (1979) Bile Pigments. In: Dolphin D. (ed.) The Porphyrins, Vol. VI. Academic Press, New York, p. 293; scrambling may be minimized by applying very dilute solutions during oxidation of **1** with *DDQ* (see p. 310 and p. 453)
[3] Manitto P., Monti D. (1974) Gazz. Chim. Ital. **104**: 513
[4] Bonnett R., McDonagh A. F. (1970) J. Chem. Soc. Chem. Comm. **1970**: 238
[5] Cole W. J., Chapman D. J., Siegelman H. W. (1968) Biochemistry **7**: 2929; Gray C. H., Lichtarowicz-Kulczycka A., Nicholson D. C., Petryka Z. (1961) J. Chem. Soc. **1961**: 2264; Nichol A. W., Morell D. B. (1969) Biochim. Biophys. Acta **177**: 599
[6] Addition of methanol to the vinyl groups does not occur with the biliverdin system as has been erroneously stated in ref. [3]; see, however, Lehner H., Riemer W., Schaffner K. (1979) Liebigs Ann. Chem. **1979**: 1798
[7] Lehner H., Braslavsky S., Schaffner K. (1978) Liebigs Ann. Chem. **1978**: 1990
[8] Krois D., Lehner H. (unpublished observations)
[9] Krois D., Lehner H. (in press) J. Chem. Soc. Perkin Trans. I; (in press) J. Chem. Soc. Perkin Trans. II
[10] A prerequisite for the effective separation of the biliverdin esters **2b–4b** from the bridged compounds **5a/5b** and **6a/6b** is that migration of the individual diastereoisomers **a** and **b** is as slow to allow for their complete thermal equilibration during elution. Otherwise prohibitive tailing occurs. The chiral diastereoisomers **5a** and **5b**, and **6a** and **6b** may be separated by preparative t.l.c. at temperatures below $-10°C$ [9]
[11] The energy barriers to helix inversion for diastereoisomers **6** (**a** and **b**) have been estimated to amount to 89 and 87 $kJ\,mol^{-1}$, respectively (293 K) [12]
[12] Krois D., Lehner H. (in press) Monatsh. Chem. **120**
[13] Clearly, scrambling in a symmetrical substituted bilirubin does not lead to isomerization
[14] The third possible diastereoisomer possessing differently configurated chirality centres is likewise formed but in very low yields ($<2\%$) [9]
[15] Haidl E., Krois D., Lehner H. (1985) J. Chem. Soc. Perkin Trans. II **1985**: 421
[16] McDonagh A. F., Assisi F. (1972) J. Chem. Soc. Chem. Comm. **1972**: 117; Defoin-Straatmann R., Defoin A., Kuhn H. J., Schaffner K. (1982) Liebigs Ann. Chem. **1982**: 1759

Received November 8, 1988. Accepted December 5, 1988