N_{21},N_{22}-Carbonyl-bridged Biliverdin. Red-blue Color Change Effected by Conformation

Stefan E. Boiadjiev and David A. Lightner*

Department of Chemistry
University of Nevada, Reno, Nevada 89557
email: lightner@scs.unr.edu

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An N_{21},N_{22}-carbonyl-bridged mesobiliverdin, prepared in high yield by reaction of the unbridged parent (λ_{max} 639 nm, ε 15,700, chloroform) with 1,1'-carbonyldiimidazole and 1,8-diazabicyclo[5.4.0]undec-7-ene, gave magenta-colored solutions in chloroform that absorb strongly in the visible spectrum (λ_{max} 534 nm, ε 27,700) and shifted to bright blue (λ_{max} 669 nm, ε 35,300) upon addition of trifluoroacetic acid.

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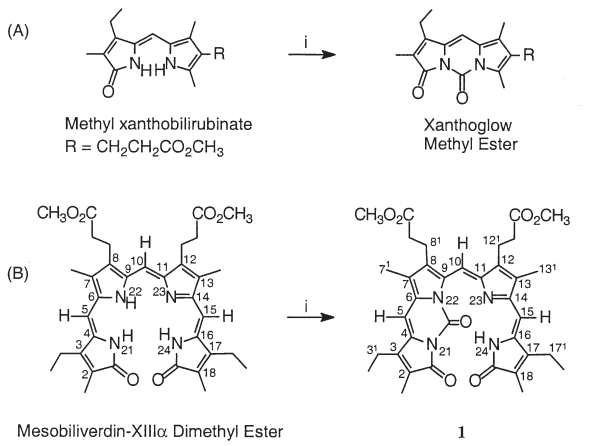
Reaction of dipyrrinones, such as methyl xanthobilirubinate (Scheme 1A) with 1,1'-carbonyldiimidazole (*CDI*) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (*DBU*) has been shown to give the N,N'-carbonyl-bridged dipyrrinones in excellent yield [1]. The latter are highly fluorescent (ϕ_F ~ 0.7-0.8) [2] but the long wavelength absorption λ_{max} (428 nm, chloroform) shows a 20 nm bathochromic shift from that of the parent (λ_{max} 406 nm, chloroform) [1,3]. A similar reaction with blue-colored (λ_{max} 639 nm, chloroform) mesobiliverdin-XIIIα dimethyl ester (Scheme 1B) afforded the N_{21},N_{22}-bridged verdin (**1**) in 76% yield. The new "verdin" was neither blue nor green, but exhibited an intense magenta color in chloroform solution (λ_{max} 534 nm) associated with a strong hypsochromic shift relative to the parent. It was also non-fluorescent to the eye. The absence of visible fluorescence at room temperature was confirmed by instrumental measurements in chloroform and trifluoroacetic acid following independent excitation of the three uv-visible absorption bands.

The structure of **1** follows from that of its symmetric verdin precursor [4]. The ^{13}C-nmr (Table 1) of **1** confirmed the expected 35 carbons resonances that correlate with those of the precursor, and a single new carbon signal at 142.9 ppm, characteristic of the carbonyl bridge connecting two nitrogens of a dipyrrinone. If the new carbonyl group had bridged the two lactam nitrogens (N_{21} and N_{24}) or the two pyrrole nitrogens (N_{22} and N_{23}), a symmetrical structure would have resulted, with approximately only one-half the number of ^{13}C signals. (In mesobiliverdin-XIIIα dimethyl ester in chloroform-d, only 18 carbon resonances are seen (Table 1), due to a rapid prototropic shift, N_{22}-H to N_{23}, tautomerism.) The ^{13}C nmr assignments and structure were further confirmed by long range heteronuclear correlation experiment (gHMBC). In particular, the hydrogen at C(5) appeared deshielded to 6.49 ppm as it does in xanthoglow methyl ester (Scheme 1A) and is correlated (^{2}J) to the carbon signals at 132.5 ppm C(4) and 130.2 ppm C(6) and (^{3}J) to C(3) at 146.6 ppm and C(7) at 123.0 ppm. In the opposite half of the molecule, the hydrogen at C(15) is with normal chemical shift at 5.84 ppm and correlated to C(13), C(14), C(16) and C(17). The ^{2}J correlation C(15)-H to C(14) and the unique ^{3}J correlation C(13^1)-CH_3 to C(14) is interesting because it reveals that C(14) is strongly deshielded (to 169.7 ppm) relative to C(6) (130.2 ppm). The carbon and proton nmr spectral data are thus consistent with structure **1** for the new bridged verdin.

Scheme 1 [a]



[a] Reagents and conditions: i, 1,1'-carbonyldiimidazole, 1,8-diazabicyclo-[5.4.0]undec-7-ene, CH_2Cl_2, reflux.

A striking color change, from magenta to bright blue was seen when solutions of **1** were exposed to acid, *e.g.*, chloroform and dimethyl-sulfoxide solutions plus trifluoroacetic acid. The color change is readily measured spectrophotometrically, as seen in the uv-visible spectra of **1** (Figure 1) in a variety of solvents, although solvents such as tetrahydrofuran, dimethylformamide and dimethylsulfoxide require larger excess of trifluoroacetic acid than do benzene, chloroform, *etc*. No similar large wavelength changes are detected for solutions of the parent mesobiliverdin-XIIIα dimethyl ester, but the intensity of its long wavelength band is also enhanced with added trifluoroacetic acid, as is seen for **1**. The spectral shift (summarized for a wide range of organic solvents in Table 2) is completely reversible, and upon addition of triethyl amine to the trifluoroacetic acid solution, the "neutral" spectrum is restored. The acid-base spectral shift cycle has been repeated numerous times with the

Table 1

^{13}C-nmr chemical shifts [a] in deuteriochloroform, assignments of carbons in **1** and comparison with its precursor, mesobiliverdin-XIIIα dimethyl ester (**MBVDME**).

Carbon		**1**	**1** + TFA [b]	**MBVDME**	**MBVDME** + TFA [b]
1	C=O	167.4	168.1	172.4	175.4
19		172.4	174.8		
2	=C–	127.3	130.7	128.4	128.8
18		130.0	132.0		
2^1	CH_3	8.5	8.5	8.3	7.3
18^1		8.4	8.4		
3	=C–	146.6	149.0	146.6	150.1
17		146.0	149.3		
3^1	CH_2	18.0	18.1	17.8	17.7
17^1		17.7	17.8		
3^2	CH_3	13.7	13.0	14.4	14.2
17^2		14.4	13.8		
4	=C–	132.5	136.9	139.9	140.2
16		145.9	146.6		
5	=CH–	96.5	96.8	96.1	96.9
15		95.6	95.2		
6	=C–	130.2	140.1	150.0	146.1
14		169.7	156.5		
7	=C–	123.0	125.9	128.0	131.6
13		135.0	132.9		
7^1	CH_3	9.5	9.0	9.5	9.4
13^1		9.9	9.5		
8	=C–	133.7	145.9	137.5	147.0
12		143.1	149.1		
8^1	CH_2	20.5	20.5	19.8	20.2
12^1		20.1	20.1		
8^2	CH_2	33.3	32.8	35.2	34.2
12^2		35.1	34.0		
8^3	C=O	173.0	175.5	173.1	174.4
12^3		173.1	175.2		
(8)	OCH_3	51.4	52.9	51.7	52.5
(12)		51.6	53.0		
9	=C–	129.9	127.9	140.9	132.1
11		153.2	137.6		
10	=CH–	120.2	122.8	114.2	119.2
N_{21}-N_{22}	C=O	142.9	143.1	–	–

[a] In ppm downfield from $(CH_3)_4Si$ for 1 x 10^{-2} *M* solutions at 25°C. The numbering system may be found in Scheme 1B; [b] Trifluoroacetic acid.

same solution, data that indicate the robust nature of the bridged verdin.

The color change of **1** upon acidification cannot be accounted for simply in terms of protonating imine nitrogen-23 (Figure 2), as the parent unbridged verdin does not exhibit a spectral shift. The spectral shift can be accommodated by a change in pigment conformation, as shown by nuclear Overhauser effect (nOe) experiments. Thus, in deuteriochloroform, nOes are observed from the C(5)-H and the C(7^1)-CH_3 and C(3^1)-CH_2– groups, as expected for the bridged dipyrrinone component. And nOes are seen between the C(15)-H and the C(13^1)-CH_3 and C(17^1)-CH_2-, consistent with the unbridged *syn-Z*-dipyrrinone conformation shown (Figure 2). These nOes are also

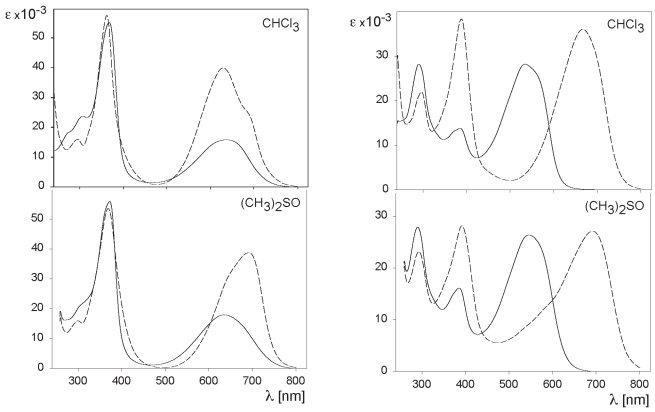


Figure 1. Uv-vis spectra of mesobiliverdin-XIIIα dimethyl ester (left) and the N_{21},N_{22}-carbonyl-bridged verdin **1** (right) in organic solvents (solid line) and with added trifluoroacetic acid (dashed line).

observed upon addition of trifluoroacetic acid. Under such conditions, nOes are also seen between the C(10)-H at 8.13 ppm and the propionic ester β-methylene groups at both C(8^1) (3.11 ppm) and C(12^1) (3.15 ppm) for the blue solution. In clear contrast, the magenta-colored neutral solution in deuteriochloroform alone shows an nOe between the C(10)-H at 7.75 ppm and only the C(12^1) β-methylene (at 2.94 ppm) and weaker to the α-methylene. These data are consistent with two different conformations for **1** (Figure 2): a rotated or stretched conformation for the magenta solution in deuteriochloroform, and a porphyrin-like conformation for the blue solution.

X-ray crystallographic studies of biliverdin dimethyl ester reveal a helical porphyrin-like conformation, with a *syn-Z* orientation around the carbon-carbon double bonds at C(4), C(10) and C(15) [5]. From the atomic coordinates, one may calculate small torsion angles: N(21)-C(4)-C(5)-C(6) = 6.5°, C(4)-C(5)-C(6)-N(22) = 11.8°, N(22)-C(9)-C(10)-C(11) = 9.6°, C(9)-C(10)-C(11)-N(23) = 2.0°, N(23)-C(14)-C(15)-C(16) = 18.5° and C(14)-C(15)-C(16)-N(24) = 3.2°. There is no reason to think that the structure of the analogous mesobiliverdin-XIIIα dimethyl ester would differ much [6]. However, color-altering distortions of the verdin structure have been engineered into the skeleton by synthesis: a *tert*-butyl group at C(10) opens the verdin helix to give red compounds with the ~640 nm long wavelength λ_{max} shifted to 490-543 nm [7,8], and a stilbene belt connecting the two lactams that forces the pigment to adopt a porphyrin-like conformation and exhibit a blue color (λ_{max} 608 nm) when *cis*-stilbene and a stretched shape with a magenta color (λ_{max} 559 nm), when *trans*-stilbene [9]. The pigment color-conformation relationship is consistent with that observed for **1**, where conformational change is induced by protonation and detected unequivocally by nOe experiments.

Table 2

Solvent dependence of the uv-visible spectral data of **1** and its mesobiliverdin-XIIIα dimethyl ester (**MBVDME**) precursor.

	ε_{max} (λ_{max}, nm) [a]			
	1	**1** + TFA [b]	**MBVDME**	**MBVDME** + TFA [b]
Benzene	27,700 (539)	33,500 (668)	16,600 (636)	46,700 (623)
	13,600 (386)	37,200 (390)	52,400 (369)	48,600 (365)
	25,800 (291)	19,200 (297)	sh 21,500 (sh 309)	13,500 (293)
Chloroform	27,700 (534)	35,300 (669)	15,700 (639)	39,800 (631)
	13,500 (385)	38,000 (388)	55,100 (369)	57,400 (363)
	27,700 (290)	21,800 (296)	23,600 (308)	15,900 (297)
Tetrahydro-	28,600 (536)	25,400 (678)	17,200 (628)	36,600 (628)
furan	15,100 (383)	28,200 (388)	54,200 (366)	46,200 (365)
	28,100 (287)	23,000 (291)	16,500 (271)	
Acetonitrile	27,200 (532)	35,400 (670)	15,800 (635)	35,100 (646)
	16,500 (380)	36,000 (387)	56,300 (363)	72,800 (351)
	28,000 (285)	23,100 (293)	sh 22,800 (sh 301)	24,800 (302)
Methanol	25,900 (536)	31,600 (667)	15,800 (643)	33,800 (688)
	15,000 (380)	33,200 (387)	55,500 (365)	56,500 (359)
	26,900 (286)	19,500 (293)	sh 16,500 (sh 270)	16,800 (299)
N-Methyl-	25,700 (542)	29,900 (684)	15,400 (647)	36,200 (690)
formamide	16,600 (384)	34,800 (393)	53,900 (369)	56,100 (365)
	27,800 (290)	21,800 (293)	sh 21,500 (sh 304)	14,700 (299)
Dimethyl-	25,800 (545)	25,600 (692)	17,900 (635)	38,800 (692)
sulfoxide	15,600 (385)	27,200 (391)	55,900 (372)	53,600 (369)
	27,100 (290)	22,600 (292)	sh 21,200 (sh 305)	15,900 (299)

[a] At 22 °C, concentration 2.3-2.4 x 10^{-5} *M*; λ in nm, ε in liters.mol^{-1}.cm^{-1}; [b] Trifluoroacetic acid, concentration ~3.6 x 10^{-2} *M*, molar ratio to pigment ~1500:1.

No evidence was found for $Z \rightarrow E$ isomerization in **1** or its verdin precursor at C(4) and C(15) as the source of the color change in **1**; the nOes from the C(5)-H and C(15)-H remain the same in deuteriochloroform and in deuteriochloroform + trifluoroacetic acid. The influence of trifluoroacetic acid on the ^{13}C-nmr chemical shifts in the verdin precursor is small for the aliphatic carbons and C(5)/C(15). The most noticeable changes occur with the sp^2-carbons: C(10) is deshielded by 5 ppm in the verdin precursor, C(7)/C(13) by ~4 ppm and C(8)/C(12) by ~ 10 ppm. In contrast C(9)/C(11) and C(6)/C(14) are strongly shielded by ~9 ppm and ~4 ppm, respectively, upon addition of trifluoroacetic acid. The behavior of **1** is qualitatively similar: the aliphatic and C(5)/C(15) carbons do not change much, but C(10) is deshielded by 3 ppm in the protonated sample. C(6) is deshielded by 10 ppm but the corresponding C(14) is shielded. Similarly C(4) and C(7) are deshielded by ~4 ppm and C(3) is deshielded by ~2 ppm. Most strikingly, C(11) and C(14) shift upfield by 15 ppm and 13 ppm, respectively, upon protonation of the sample.

Further work is in progress to investigate **1** as a probe of biological pH.

EXPERIMENTAL

Nuclear magnetic resonance (nmr) spectra were obtained on a Varian Unity Plus spectrometer operating at 500 MHz (proton) and 125 MHz (C-13) in deuteriochloroform solvent. Chemical shifts are reported in ppm referenced to the residual chloroform proton signal at 7.26 ppm and C-13 signal at 77.00 ppm unless otherwise noted. All ultraviolet-visible spectra were recorded on a Perkin-Elmer Lambda-12 spectrophotometer in 1 cm quartz cells at 20 °C. The final concentration of the solutions was 2.0-2.5 x 10^{-5} *M*. Fluorescence (or lack thereof) was checked on a Jobin Yvon Fluorolog 3 model FL3-22 instrument at 295-297 K. Melting points were taken on a Mel-Temp capillary apparatus and are uncorrected. Analytical thin layer chromatography (tlc) was carried out on J.T. Baker silica gel IB-F plates (125 µm layer). Radial chromatography was carried out on Merck preparative layer grade silica gel PF_{254} with $CaSO_4$ binder using a Chromatotron (Harrison Research, Inc., Palo Alto, CA). All solvents were reagent grade obtained from Fisher or Aldrich. Deuterated chloroform was from Cambridge Isotope Laboratories. The spectral data were obtained in spectral grade solvents and were dried and purified according to standard procedures [10].

(4*Z*,10*Z*,15*Z*)-N_{21},N_{22}-Carbonyl-3,17-diethyl-8,12-bis-(2-methoxycarbonylethyl)-2,7,13,18-tetramethyl-(21*H*,24*H*)-bilin-1,19-dione (N_{21},N_{22}-Carbonyl-mesobiliverdin-XIIIα Dimethyl Ester) (**1**).

A mixture of 246 mg (0.4 mmole) of mesobiliverdin-XIIIα dimethyl ester [4], 130 ml of anhydrous methylene chloride, 324 mg (2.0 mmoles) of 1,1'-carbonyldiimidazole (CDI), and 0.3 ml (2.0 mmoles) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was heated under nitrogen at reflux for 3 hours. Then more 324 mg (2.0 mmoles) of CDI and 0.3 ml (2.0 mmoles) of DBU were added and reflux continued for 2 hours. After cooling, the

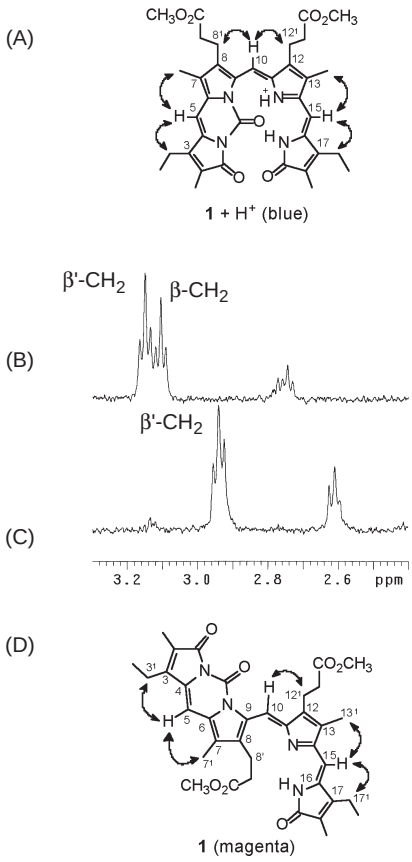


Figure 2. (A) Porphyrin-like (10-*syn*) and (D) stretched (10-*anti*) conformers **1** with nOes shown by double-headed arrows. The nOe enhancements of the propionic ester CH_2 signals on irradiation of the C(10)-H are shown in: (B) acidified deuteriochloroform and (C) neutral deuteriochloroform. Protonation in solution drives the equilibrium to the porphyrin-like conformation (top) as can be observed by the striking color change. In the porphyrin-like conformation, the propionic acid β and β'-CH_2 groups have similar chemical shifts (B) in the ^{1}H-nmr. In the stretched conformation they are more shielded and more widely separated (C).

mixture was washed with 100 ml of 1% aqueous HCl (color change of the organic phase from dark purple into deep blue), then with water (3 x 100 ml) (the color returns to saturated magenta) and the solution was dried over anhydrous sodium sulfate. After filtration and evaporation of the solvent under vacuum, the residue was purified by radial chromatography on silica gel. The fractions containing nonpolar magenta colored pigment were combined, the solvents were evaporated under vacuum and the residue was recrystallized from ethyl acetate-hexane to afford 194 mg (75%) of **1**, m.p. 205-207 °C; ^{1}H nmr (deuteriochloroform): δ 1.18 (t, 3H, 17^2-CH_3, J = 7.6 Hz), 1.24 (t, 3H, 3^2-CH_3, J = 7.6 Hz), 1.91 (s, 3H, 18^1-CH_3), 1.98 (s, 3H, 2^1-CH_3), 2.08 (s, 3H, 13^1-CH_3), 2.25 (s, 3H, 7^1-CH_3), 2.41 (t, 2H, 8^2-CH_2, J = 7.5 Hz), 2.48 (q, 2H, 17^1-CH_2, J = 7.6 Hz), 2.57 (q, 2H, 3^1-CH_2, J = 7.6 Hz), 2.61 (t, 2H, 12^2-CH_2, J = 7.8 Hz), 2.94 (t, 2H, 12^1-CH_2, J = 7.8 Hz), 3.13 (t, 2H, 8^1-CH_2, J = 7.5 Hz), 3.53 (s, 3H, (8)-OCH_3), 3.67 (s, 3H, (12)-OCH_3), 5.84 (s, 1H, 15-CH=), 6.49 (s, 1H, 5-CH=), 7.75 (s, 1H, 10-CH=), 10.26 (br.s, 1H, 24-NH) ppm.

Anal. Calcd. for $C_{36}H_{40}N_4O_7$ (640.7): C, 67.48; H, 6.29; N, 8.74. Found: C, 67.59; H, 6.30; N, 8.91.

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