Regioselective Porphyrin Bridge Cleavage Controlled by Electronic Effects. Coupled Oxidation of 3-Demethyl-3-(trifluoromethyl)mesohemin IX and Identification of Its Four Biliverdin Derivatives†

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This report describes the nonenzymatic oxidative cleavage of the title porphyrin (**2**) performed with oxygen and ascorbic acid in aqueous pyridine at 37 °C (coupled oxidation), via hydrolysis of the corresponding verdoheme intermediates, followed by esterification of the resulting free acid mesobiliverdin analogues to their dimethyl esters **4** (α isomer), **5** (*β* isomer), **6** (*γ* isomer), and **7** (*δ* isomer). The four biliverdin derivatives were purified by HPLC, and their structures were confirmed by FAB MS and also by UV−vis and ^{1}H NMR spectroscopies. The purity of each compound was checked by ^{19}F NMR, and the four regioisomers were assigned through their 2D-NMR ROESY spectra and confirmed by UV−vis spectroscopy. The ratio of regioisomers was determined by ^{19}F NMR spectroscopy before any purification of single compounds was attempted: α:*β*:*γ*:*δ* 11:6:26:57 (%). This unusually high regioselectivity was attributed to the electron-withdrawing effect of the CF_3 group on the electronic structure of porphyrin as shown considering the ab initio calculations of an iron(II) *β*-substituted (trifluoromethyl)porphyrin used as a model compound. In porphyrin **2**, the oxidation clearly takes place at the electron richest *meso* positions, the order of reactivity strictly following that of electron density, pointing out that the regiospecificity of the bridge cleavage can be effectively controlled by the electronic effects of some strategic substituents in the chromophore. The relevance of all these results in the study of the mechanism of the reactions involved in the natural catabolism of heme, catalyzed by heme oxygenase, is discussed. The advantages of **2** derived from this work, which make it a suitable model compound for the enzymatic reaction, are also discussed.

Introduction

In vivo degradation of heme, catalyzed by heme oxygenase, is based on the porphyrin susceptibility to oxidative attack at the *meso* positions,[1] under very mild conditions, with elimination of the α-carbon bridge as a molecule of CO, resulting in the cleavage of the tetrapyrrolic macrocycle and formation of biliverdin IXα.[2] In mammal catabolism, biliverdin IXα is then reduced to bilirubin IXα by biliverdin reductase[3] (Scheme 1). In pyridine, hemin (**1**) is also readily cleaved by treatment with oxygen in the presence of ascorbic acid as a reducing agent. This process, the so-called coupled oxidation, has been widely used as a model of the heme oxygenase catalyzed reaction.[3,4]

Due to the unsymmetrical disposition of the substituents in the natural protoporphyrin IX, four different biliverdins can a priori be obtained. The natural α specificity in the enzymatic process is of great significance due to the unique chemical properties of bilirubin IXα compared to its regioisomers, i.e., its lipophilicity that makes it unexcretable and potentially toxic.[5] The origin of the α selectivity in the natural catabolism of heme is still under discussion.

Perfluoroalkyl-substituted porphyrins have recently received much interest due to their unique redox properties and the advantage of using ^{19}F as an NMR probe.[6] On the other hand, only this year the first fluorinated bile pigment has been described.[7] To gain more understanding in the regioselectivity of the biochemical reaction, as well as to increase knowledge about the reactivity and properties of fluorinated pyrrole pigments, here we report the results on the coupled oxidation of the electron-deficient porphyrin **2** (Chart I).[8] We describe the iden-

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(1) For a detailed description of pyrrole pigment nomenclature, see: Nomenclature of Tetrapyrroles, Recommendations of 1986, International Union of Pure and Applied Chemistry and International Union of Biochemistry, Joint Commission on Biochemical Nomenclature. *Pure Appl. Chem.* **1987**, *59*, 779−832.

(2) (a) Maines, M. D. *Heme Oxygenase: Clinical Applications and Functions*; CRC Press: Boca Raton, FL, 1992. (b) O'Carra, P. Heme-Cleavage: Biological Systems and Chemical Analogues. *Porphyrins and Metalloporphyrins*; Smith, K. M., Ed.; Elsevier Scientific Publishing Co.: Amsterdam; 1975; pp 123−153.

(3) Schmid, R.; McDonagh, A. F. In *The Porphyrins*; Dolphin, D., Eds.; Academic Press: New York, 1979; Vol. 6, pp 257−292.

(4) Bonnett, R.; McDonagh, A. F. *J. Chem. Soc., Perkin Trans. 1* **1973**, 881−888.

(5) Lightner, D. A.; McDonagh, A. F. *Acc. Chem. Res.* **1984**, *17*, 417−424.

(6) Suzuki, A.; Tomizawa, T.; Hayashi, T.; Mizutani, T.; Ogoshi, H. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2923−2933.

(7) Boiadjiev, S. E.; Lightner, D. A. *J. Org. Chem.* **1997**, *62*, 399−404.

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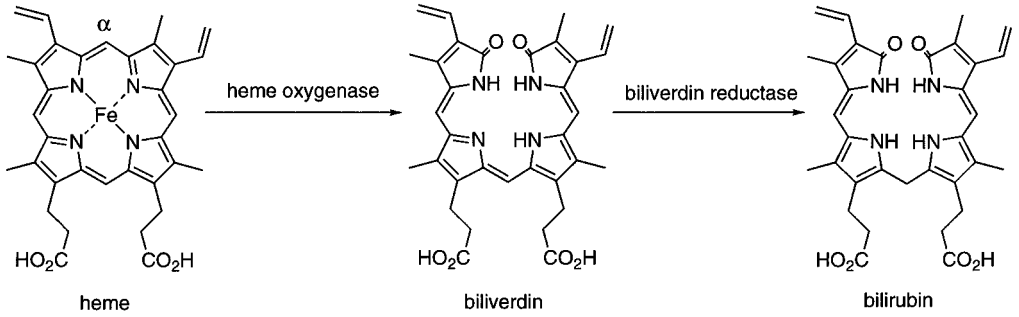
Scheme 1



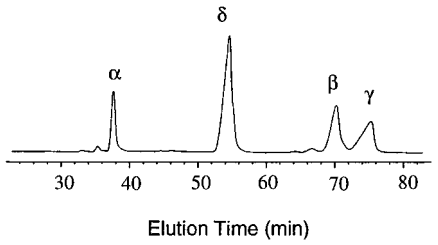
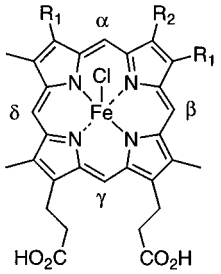


Figure 1. Semipreparative HPLC chromatogram of a mixture of the dimethyl esters **4**, **5**, **6**, and **7** obtained in the coupled oxidation of porphyrin **2**. Each isomer is labeled according to the *meso* carbon extruded as a molecule of CO. See the Experimental Section for the chromatographic conditions.

Chart 1



	R_1	R_2
1	vinyl	CH_3
2	ethyl	CF_3
3	ethyl	CH_3

tification of the four biliverdin isomers using the advantages of ^{19}F NMR spectroscopy and the determination of the regioselectivity through their ratios and discuss these results in perspective with previously published papers.

Results

Obtention, Isolation, and Identification of the Four Mesobiliverdin Dimethyl Ester Derivatives. The nonenzymatic oxidative cleavage of porphyrin **2** was performed with oxygen−ascorbic acid in aqueous pyridine at 37 °C via hydrolysis of the verdoheme intermediates[9] followed by esterification of the resulting free acid mesobiliverdin analogues to their dimethyl esters (**4**, **5**, **6**, and **7**, Chart 2), according to well-established experimental procedures.[4] The obtained substances were isolated by reverse-phase HPLC, as shown in Figure 1. Each substance gave a single ^{19}F NMR signal and a satisfactory FAB peak confirming that the reaction proceeded to the four expected mesobiliverdin compounds. The isomers were differentiated by means of the nuclear Overhauser effect (NOE) of the methine protons with their proximal β-substituents, measured by 2D ROESY ^1H NMR spectroscopy. The spectra are presented in Figure 2. The associated NOE of the signals appearing at lower fields (6.8−6.6 ppm), which were assigned to the central methine bridge protons of each molecule on the basis of the ROESY correlations, can be used as an unambiguous proof of isomer constitution. The results are consistent as a whole, providing an unequivocal assignment of each regioisomer and thus allowing the matching of each HPLC band and ^{19}F NMR signal to its corresponding compound.

UV−Vis Spectra. The UV−vis spectra of compounds **4**, **5**, **6**, and **7** in CH_2Cl_2 are shown in Figure 3. There is a significant difference in the values of the absorption maxima of each regioisomer. Up to 20 nm in the high energy band and more than 35 nm in the wider band at lower energies were detected. Several different factors influence the light absorption properties of biliverdins, e.g., the overall conformation of the pigment and the electronic properties of the substituents.[10] The differences in the spectra of Figure 3 can be mainly attributed to the different electronic effects caused by the relative position of the strong electron-withdrawing CF_3 group within the biliverdin framework. This hypothesis is reasonable to assume, since their nonfluorinated counterparts present only slight differences in their absorption maxima (about 5 nm in the narrow bands at higher energies[4,11]). Furthermore, the configuration and conformation around exocyclic double bonds in **4**, **5**, **6**, and **7** are the same in all isomers (helicoidal *Z,syn*, *Z,syn*, *Z,syn* as seen by the NOE described above) and the general conformation differences among them cannot be significantly greater than those in their nonfluorinated counterparts. This is suggested by the similar intensity ratios of the two absorption bands in each isomer and the fact that the relatively small volume of the CF_3 group is not expected to exert large conformational changes due to steric interactions.

Quantum mechanical calculations of the Pariser−Parr−Pople (PPP) type[12] show that the absorption bands of biliverdins with an electron-withdrawing substituent at a terminal ring (positions 2 and 3) present a large bathochromic shift compared to those bearing the same substituent in a central ring (positions 7 and 8). This

(8) Toi, H.; Homma, M.; Suzuki, A.: Ogoshi, H. *J. Chem. Soc., Chem. Commun.* **1985**, 1791−1792.

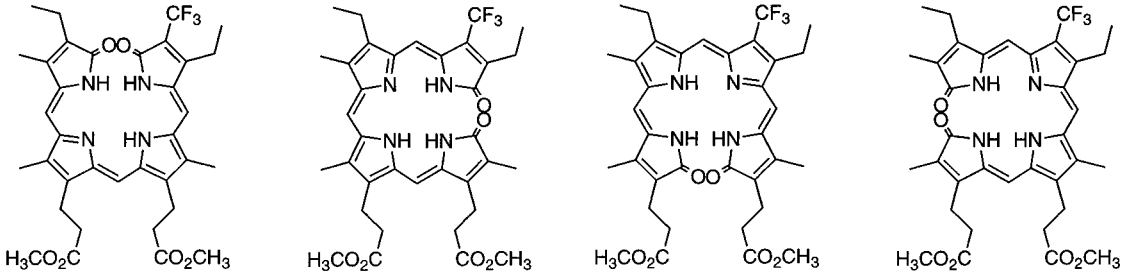
(9) The mixture of verdohemes was used as obtained without further purification or isolation. However, the UV−vis spectrum in aqueous pyridine was in agreement with those of iron oxaporphyrins, and the FABMS [(+), 3-nitrobenzyl alcohol] showed an intense peak at m/z = 677 which accounts for the mass of the fluorinated mesoverdoheme cations M^+ $[C_{33}H_{32}F_3Fe^{II}N_4O_5]^+$.

(10) Falk, H. *The Chemistry of Linear Oligopyrroles and Bile Pigments*; Springer-Verlag: Wien, New York, 1989; pp 401−423.

(11) McDonagh, A. F. In *The Porphyrins*; Dolphin, D., Eds.; Academic Press: New York, 1979; Vol. 6, pp 293−491.

(12) Tokita, S.; Matsuoka, K.; Kogo, T.; Kihara, K. *Molecular Design of Functional Dyes − PPP Method and its Applications*; Maruzen, Tokyo, 1990. Unpublished results.

Chart 2



4 α isomer **5** β isomer **6** γ isomer **7** δ isomer

Table 1. Regioisomer Distribution in the Coupled Oxidation of Heme Derivatives

	isomer, % (ratio)				
porphyrin	α	β	γ	δ	ref
hemin (**1**)	32 (1.6)	25 (1.2)	23 (1.1)	20 (1)	*b*[c]
trifluoromesohemin (**2**)	11 (1.8)	6 (1)	26 (4.5)	57 (9.5)	this work[a]
mesohemin (**3**)	33 (1.9)	26 (1.5)	17 (1)	24 (1.4)	*b*

[a] Approximate error in the distribution percentage due to signal integration was ±2%. [b] Reference 2b. [c] Using the same experimental conditions as for **2** we could detect an isomer distribution ratio of 1.6:1.5:1.1:1 by HPLC, although the isomers were not identified, clearly in contrast with the results for **2**.

Table 2. Atomic Charges for [2,3,7,8,12,13,17-Heptamethyl-18-(trifluoromethyl)porphyrinato]iron(II). Spin Multiplicity: Singlet

position		atomic charges from electrostatic potential	Mulliken population analysis	natural atomic orbital populations[a]
δ	(C−5)	−0.432623	−0.379766	−0.371412
γ	(C−10)	−0.368814	−0.360774	−0.328568
α	(C−20)	−0.261714	−0.310751	−0.229719
β	(C−15)	−0.181116	−0.284706	−0.165160

[a] Löwdin, P. O. *Phys. Rev.* **1955**, *97*, 1474. Reed, A. E.; Weinstock, R. B.; Weinhold, F. *J. Chem. Phys.* **1985**, *83*, 735.

effect is more pronounced in the band absorbing at lower energies. The same calculations clearly show that the most blue-shifted low-energy band is associated with the isomer bearing the electron-withdrawing group at C7, in this case, corresponding to the δ isomer. These calculations lead us to attribute each spectrum to one exclusive isomer, thus supporting the assignments done by NMR spectroscopy.

The UV−vis results shown in Figure 3 provide a sensitive way to differentiate each regioisomer, support the assignments done by means of the NOE, and illustrate the relative susceptibility of each position in the biliverdin skeleton toward the electron-withdrawing effects of the substituents.

Regioisomer Distribution in the Coupled Oxidation of Porphyrin 2. The dimethyl esters of the mesobiliverdin regioisomers gave a well-resolved ^{19}F NMR signal. The intensity of each signal allowed us to quantify the relative abundance of each compound before any purification of single substances was attempted.[13] The results are presented in Table 1.

The introduction of the trifluoromethyl group causes the nonenzymatic oxidative cleavage of the porphyrin to proceed with a great degree of regioselectivity compared to the almost nonspecific cleavage in normal heme. This result is attributed to the electron-withdrawing effect of the CF_3 group on the electronic structure of the porphyrin. The preferency for certain *meso* positions in the bridge cleavage process can be easily rationalized considering the ab initio calculations[14] of an iron(II) β-substituted (trifluoromethyl)porphyrin used here as a simple model compound (Table 2). Clearly, in porphyrin **2** the oxidation takes place at the electron richest *meso* positions. The order of reactivity strictly follows that of electron density. Our results are in agreement with similar conclusions derived from previously published calculations on normal heme.[15] This allows accurate interpretation of the regioselectivity of the process by considering the electronic structure of the porphyrin.

Discussion

The heme oxygenase catalyzed cleavage of heme is thought to proceed in a three-step way via formation of α-*meso*-hydroxyheme, aerobic fragmentation of this α-*meso*-hydroxyheme to give verdoheme, and subsequent oxidative cleavage to biliverdin (Scheme 2).[2a,16] In a first stage, the enzyme-bound hemin is reduced by cytochrome P450 reductase/NADPH to the ferrous form which binds oxygen. A second electron is then accepted by the ferrous dioxygen complex resulting in the activated form and subsequent regioselective attack on the porphyrin ring.[17] The mechanism of this first oxygenation step is the least clear in the heme degradation sequence. To elucidate this mechanism, more experimental work is needed. It has already been shown that this involves an intramolecular addition of the iron bound peroxide (activated species) to the porphyrin ring.[18] Based on results obtained with ethyl hydroperoxide and heme oxygenase, it has been reported that an electrophilic (or possibly radical but not nucleophilic) attack is involved.[19]

(13) It is known that some published results are incorrect due to preferential isolation of isomers during workup steps. See ref 2b and references cited therein.

(14) The calculations were carried out with Spartan version 3.1.2., Wavefunction Inc.

(15) Pullman, B.; Perault, A.-M. *Proc. Natl. Acad. Sci. U.S.A.* **1959**, *45*, 1476−1480. However, in normal heme the electronic effects caused by the substituents are not strong enough to show a pronounced regioselectivity in the coupled oxidation process in the absence of the protein.

(16) Wilks, A.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1993**, *268*, 22357−22362 and references therein.

(17) Yoshida, T.; Noguchi, M.; Kikuchi, G. *J. Biol. Chem.* **1980**, *255*, 4418−4420.

(18) Tajima, K.; Tada, K.; Shigematsu, M.; Kanaori, K.; Azuma, N.; Makino, K. *J. Chem. Soc., Chem. Commun.* **1997**, 1069−1070.

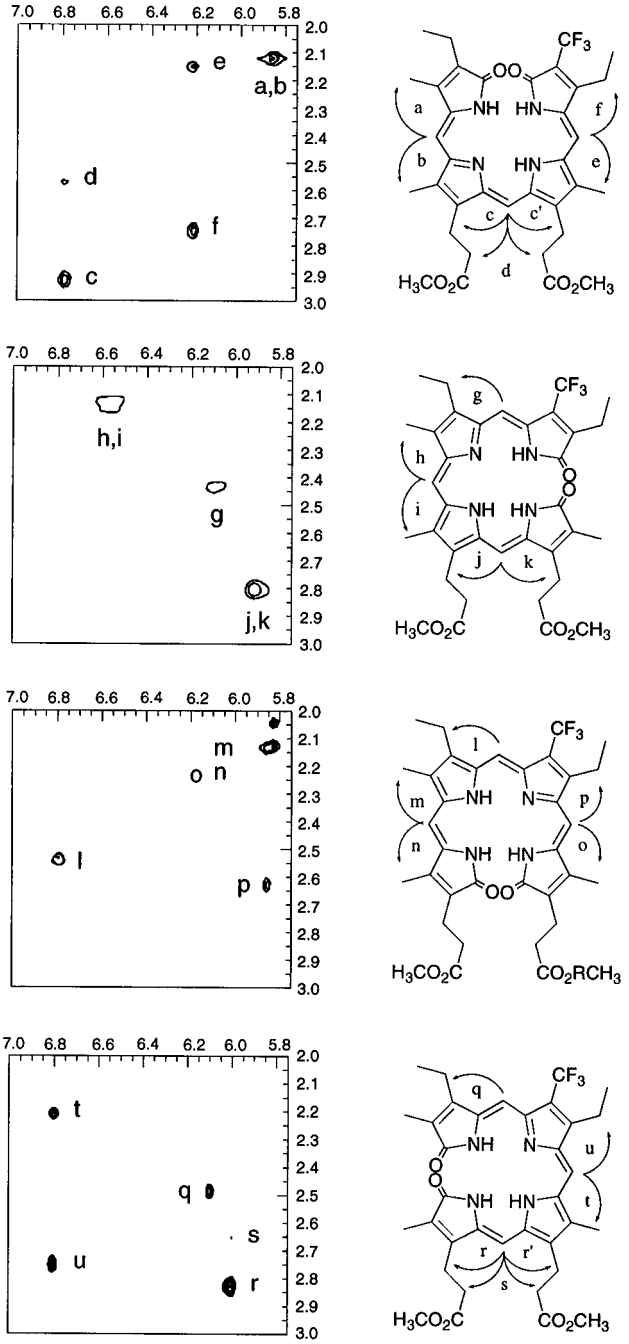


Figure 2. ROESY cross peaks of the methine protons of compounds **4**, **5**, **6**, and **7** used to identify each regioisomer. The spectra were recorded in $CDCl_3$. Each cross peak is assigned as shown with its label.

The origin of the regioselectivity in the hydroperoxide attack on the α-*meso* carbon in the natural process is thought to result from electronic and steric interactions with the enzyme environment.[20] However the ultimate details are still unknown. Although there is evidence that the first oxygenation step is not uniquely responsible

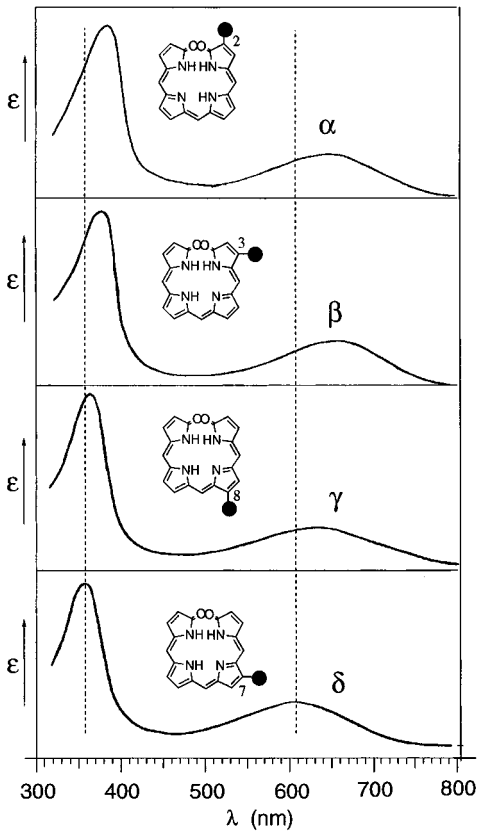


Figure 3. UV−vis spectra of compounds **4**, **5**, **6**, and **7**. The spectra are recorded in a CH_2Cl_2 solution presenting an absorbance in the range of 0.9−1.1 (cell path 1 cm) and are presented in arbitrary units. The intensities are normalized in respect to the band at higher energies. The dashed lines correspond to the maxima of the δ isomer for the sake of comparison. The inset shows the position of the CF_3 electron-withdrawing group within the biliverdin framework.

for the α-selectivity,[21] it has to be the driving force in the natural systems as only the α-*meso*-hydroxy isomer is formed. Initially it was suggested that the selectivity was due to intrinsic electronic effects of the chromophore.[2b] This idea was abandoned when it was finally proved that the coupled oxidation of hemin (**1**), in the absence of the protein environment, gave a mixture of isomers presenting a random cleavage at the methine bridges.[4] It was then suggested that the protein cavity, showing regions of different hydrophobicity, influenced the reactivity of the four *meso* positions directing the oxygen attack.[22] Steric hindrance and relative accessibilities of the four methine bridges have also been questioned in order to explain the selectivity.[23] More recently it has been reported that the protein appears to exert an electronic influence on the porphyrin, giving electronic density to the α bridge, as well as causing a steric effect on the bound ligand.[21a] On the basis of results with selectively electron-enriched methine bridges in methyl-substituted mesohemin analogues, Ortiz de Montellano recently suggested that the regioselectivity in heme cleavage by

(19) Wilks, A.; Torpey, J.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1994**, *269*, 29553−29556.

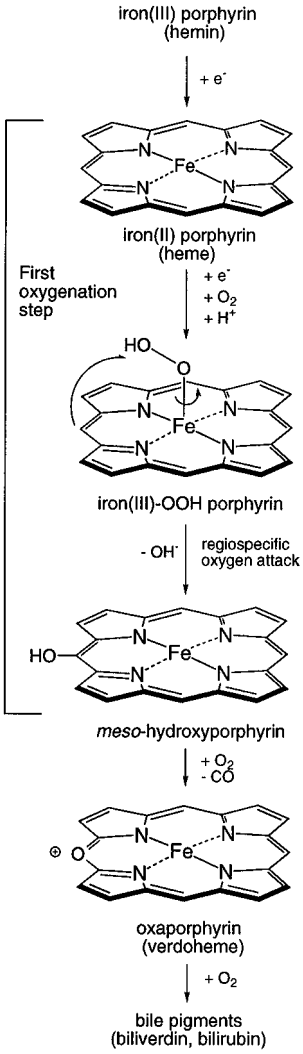
(20) (a) Hernández, G.; Wilks, A.; Paolesse, R.; Smith, K. M.; Ortiz de Montellano, P. R.; La Mar, G. M. *Biochemistry* **1994**, *33*, 6631−6641. (b) Takahashi, S.; Matera, K. M.; Fujii, H.; Zhou, H.; Ishikawa, K.; Yoshida, T.; Ikeda-Saito, M.; Rousseau, D. L. *Biochemistry* **1997**, *36*, 1402−1410. (c) Takahashi, S.; Ishikawa, K.; Takeuchi, N.; Ikeda-Saito, M.: Yoshida, T.; Rousseau, D. L. *J. Am. Chem. Soc.* **1995**, *117*, 6002−6006.

(21) Of the four synthetic *meso*-hydroxyhemes only the α-isomer was efficiently converted to biliverdin by heme oxygenase. However, the fate of the δ-, γ-, and β-*meso*-hydroxy isomers of other porphyrins formed in situ is not clear. See: Yoshinaga, T.; Sudo, Y.; Sano, S. *Biochem. J.* **1990**, *270*, 659−664.

(22) O'Carra, P.; Colleran, A. *FEBS Lett.* **1969**, *5*, 295−298.

(23) Brown, S. B.; Chabot, A. A.; Enderby, E. A.; North, A. C. T. *Nature* **1981**, *289*, 93−95.

Scheme 2



heme oxygenase is controlled by electronic rather than steric effects.[24] Notwithstanding in that case the mechanism proceeds via an alternative pathway not involving the α-*meso*-hydroxylated species.

The results on the electron-density-dependent regioselective cleavage of porphyrin **2** described in the previous section led us to conclude that in the first oxygenation step of the heme oxygenase catalyzed sequence there is, indeed, an apparent electrophilic attack of the iron bound activated oxygen. However, the results described here do not allow us to unequivocally conclude whether there is an intramolecular electron transfer with homolytic scission of the O−O bond and subsequent radical attack to the metalloporphyrin π radical cation or a pure electrophilic process through porphyrin carbocations. Such ambiguity remains because both processes would preferentially occur at the electron richest *meso* positions. Nevertheless, following the coupled oxidation reaction of porphyrins, the attack is performed by hydroperoxide itself and proceeds through the normal route involving the *meso*-hydroxyporphyrins.[3,4] Thus, our model system mimics the natural mechanism to a great extent. The same results also point out that the regiospecificity of the bridge cleavage can be effectively controlled by the electronic effects of some strategic substituents in the chromophore.

To clarify the relative role of the electronic effects exerted by the enzyme in the α-selectivity and those of steric and hydrophobic interactions, suitable porphyrins that can be potential substrates for the enzyme,[25] which also present strong intrinsic electronic effects directing the oxygen attack to other positions than the α-bridge, are clearly needed.[27] The strong electronic effects in porphyrin **2**, along with the unequivocal and easy identification of the four biliverdin derivatives through ^{19}F NMR spectroscopy described herein, make of **2** a good candidate for such a task.

Conclusions

In summary, we have shown that the title porphyrin presents a remarkable regioselectivity in its coupled oxidation process. This regioselectivity can be directly correlated to its electronic structure. These results clearly prove that the oxidative cleavage preferentially occurs at the electron richest bridge pointing out to an electrophilic oxygen addition to the porphyrin ring. The four biliverdin derivatives resulting from the oxidative cleavage have been characterized and can be sensitively and unequivocally identified by ^{19}F NMR spectroscopy. The same porphyrin can thus constitute a suitable model for the study of the catabolism of natural porphyrins presenting an electron deficiency at the α *meso* bridge, such as Spirographis hemin.[26b] According to all these results, porphyrin **2** constitutes a good candidate to test the influence of the intrinsic electronic effects of the chromophore in the natural heme oxygenase catalyzed reaction. In this respect, more work is in progress.

Experimental Section

General. ^1H and ^{19}F NMR spectra were recorded on a JEOL A-500 FT NMR spectrometer, and chemical shifts are referenced to internal $CDCl_3$ (^1H δ 7.24 ppm downfield) relative to Me_4Si at 0 ppm or downfield to $CFCl_3$ (^{19}F δ 0 ppm). Data are recorded as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), relative intensities, and identification. For ^{19}F NMR, a 60° pulse angle and a 2.2-s relaxation delay, which was longer than 10 T_1, were used.[28] Two-dimensional phase-sensitive ROESY spectra were obtained with a mixing time of 200 ms. UV−vis

(24) Torpey, J.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1996**, *271*, 26067−26073. Some of the conclusions in that paper are based on the undetection of 10-methyl-substituted biliverdins. It is described that such kind of biliverdins readily tautomerize to a bilirubinoid structure that presents different absorption and chromatographic properties. For examples see ref 10, pp 245−246.

(25) Frydman and Frydman described the structural requirements of the substrate involved in the specificity of heme oxygenase, the presence of the vicinal propionic acid chains being the most important requirement (see ref 26). Electron-withdrawing groups at the β-position of rings A and B decreased substrate activity but did not suppress it.

(26) (a) Frydman, R. B.; Tomaro, M. L.; Buldain, G.; Awruch, Díaz, J. L.; Frydman, B. *Biochemistry* **1981**, *20*, 5177−5182. (b) Tomaro, M. L.; Frydman, R. B.; Frydman, B.; Pandey, R. K.; Smith, K. M. *Biochim. Biophys. Acta* **1984**, *791*, 342−349.

(27) It has been described that although deuterohemin is believed to direct the oxidation to the δ-bridge (see ref 2b) the α specificity imposed by the enzyme prevails, see ref 29. These results were based on the undetection of ^{14}C substituted bilirubins when [α-^{14}C]deuterohemin or even 2,4-diacetyl[α-^{14}C]deuterohemin were used as substrates. Nevertheless, due to the tremendous solubility differences among the bilirubin regioisomers and the need of extraction and chromatography procedures in the workup, it is probably difficult to detect small amounts of the unnatural isomers. Porphyrin **2** has the potential advantage that, thanks to characterization at the biliverdin stage by ^{19}F NMR spectroscopy as described in this paper, those products can be easily determined and quantified without any purification.

spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer with a thermostated cell compartment. Mass spectra were obtained with a JEOL JMS-SX-102A mass spectrometer. HPLC analysis and purifications were performed on a Waters Model 600E multisolvent delivery system equipped with a Waters 996 photodiode array detector.

Coupled Oxidation. Following the general methodology of Bonnett and McDonagh,[4] in a typical experiment, 20 mg (28.2 μmol) of porphyrin **2** furnished 8.2 mg (12.2 μmol) of a mixture of the dimethyl esters **4**, **5**, **6**, and **7** (43% from the starting metalloporphyrin) along with small amounts of unidentified nonverdinoid pigments, as detected by TLC. At this stage of the synthetic procedure, the ratio of regioisomers was determined by ^{19}F NMR spectroscopy. The mixture was then purified by HPLC, and each single major compound was identified as described below.

HPLC Purification and Identification of the Four Demethyl(trifluoromethyl)mesobiliverdin Dimethyl Ester Constitutional Isomers. The crude reaction mixture was purified by reverse-phase HPLC on a YMC-PACK ODS AQ, SH-343-5AQ S-5 μm 120A 250 × 20 mm i.d. semipreparative reverse-phase column eluted with MeOH:H_2O 85:15 (v:v) at a flow rate of 8 mL min^{-1} (≈900 psi). All solvents were HPLC grade and were carefully degassed prior to use. At time zero, the sample was injected. The elution profile was monitored at $\lambda = 365$ nm. The central part of each major band was collected and analyzed. The following compounds were characterized (not in order of elution).

(4*Z*,9*Z*,15*Z*)-2,17-Diethyl-1,19,23,24-tetrahydro-3,7,13-trimethyl-1,19-dioxo-18-(trifluoromethyl)-21*H*-bilin-8,12-dipropionic acid dimethyl ester (4, α isomer): a blue solid; TLC R_f 0.74 ($CHCl_3$:acetone 3:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.80 (s, 1H, C10-H), 6.23 (s, 1H, C15-H), 5.88 (s, 1H, C5-H), 3,66 and 3.65 (2s, 2 × 3H, CO_2CH_3), 2.94−2.89 (m, 4H, C*H*$_2$CH$_2$COO), 2.74 (q, 2H, C17-C*H*$_2$CH$_3$), 2.55 (t, 4H, CH$_2$-C*H*$_2$COO), 2.28 (q, 2H, C2-C*H*$_2$CH$_3$), 2.11 (s, 3H, C13-CH$_3$), 2.08 and 2.07 (2s, 2 × 3H, C3 and C7-CH$_3$), 1.31 (t, 3H, C17-CH$_2$C*H*$_3$) and 1.05 (t, 3H, C2-CH$_2$C*H*$_3$); ^{19}F NMR (470 MHz, $CDCl_3$) δ −60.14 (s); UV−vis (CH_2Cl_2) λ_{max} [nm] (I_{rel}) 378 (3.94), 652 (1); FABMS [(+), 3-nitrobenzyl alcohol] *m*/*z* 669 (M + 1).

(4*Z*,9*Z*,15*Z*)-2,7-Diethyl-1,19,23,24-tetrahydro-8,12,18-trimethyl-1,19-dioxo-3-(trifluoromethyl)-21*H*-bilin-13,17-dipropionic acid dimethyl ester (5, β isomer): a blue solid; TLC R_f 0.76 ($CHCl_3$:acetone 3:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.64 (s, 1H, C10-H), 6.13 (s, 1H, C5-H), 5.94 (s, 1H, C15-H), 3.69 and 3.65 (s, 3H, CO_2CH_3), 2.83−2.77 (m, 4H, C*H*$_2$CH$_2$COO), 2.63 and 2.48 (2t, 2 × 2H, CH$_2$C*H*$_2$COO), 2.46−2.41 (2q, 2 × 2H, C*H*$_2$CH$_3$) 2.16 and 2.13 (2s, 2 × 3H, C8 and C12-CH$_3$), 1.81 (s, 3H, C18-CH$_3$), 1.10 and 1.08 (2t, 2 × 3H, CH$_2$C*H*$_3$); ^{19}F NMR (470 MHz, $CDCl_3$) δ −57.97 (s); UV−vis (CH_2Cl_2) λ_{max} [nm] (I_{rel}) 378 (3.68), 662 (1); FABMS [(+), 3-nitrobenzyl alcohol] *m*/*z* 669 (M + 1).

(4*Z*,9*Z*,15*Z*)-7,12-Diethyl-1,19,23,24-tetrahydro-3,13,17-trimethyl-1,19-dioxo-8-(trifluoromethyl)-21*H*-bilin-2,18-dipropionic acid dimethyl ester (6, γ isomer): a blue solid; TLC R_f 0.80 ($CHCl_3$:acetone 3:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.83 (s, 1H, C10-H), 5.87 (s, 1H, C5-H), 5.84 (s, 1H, C15-H), 3.65 and 3.64 (2s, 2 × 3H, CO_2CH_3), 2.62 (q, 2H, C7-C*H*$_2$CH$_3$), 2.60−2.50 (m, 10H, C*H*$_2$C*H*$_2$CO$_2$ and C12-C*H*$_2$CH$_3$) , 2.12 (s, 3H, C3-CH$_3$), 2.11 (s, 3H, C17-CH$_3$), 2.02 (s, 3H, C13-CH$_3$), 1.16 and 1.13 (2t, 2 × 3H, CH$_2$C*H*$_3$); ^{19}F NMR (470 MHz, $CDCl_3$) δ −54.90 (s); UV−vis (CH_2Cl_2) λ_{max} [nm] (I_{rel}) 362 (4.33), 636 (1); FABMS [(+), 3-nitrobenzyl alcohol] *m*/*z* 669 (M + 1).

(4*Z*,9*Z*,15*Z*)-3,8-Diethyl-1,19,23,24-tetrahydro-2,12,18-trimethyl-1,19-dioxo-7-(trifluoromethyl)-21*H*-bilin-13,17-dipropionic acid dimethyl ester (7, δ isomer): a blue solid; TLC R_f 0.77 ($CHCl_3$:acetone 3:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.82 (s, 1H, C10-H), 6.12 (s, 1H, C5-H), 6.03 (s, 1H, C15-H), 3.70 and 3.67 (2s, 2 × 3H, CO_2CH_3), 2.85 and 2.83 (2t, 2 × 2H, C*H*$_2$CH$_2$CO$_2$), 2.77 (q, 2H, C8-C*H*$_2$CH$_3$), 2.65 and 2.51 (2t, 2 × 2H, CH$_2$C*H*$_2$CO$_2$), 2.50 (q, 2H, C3-C*H*$_2$CH$_3$), 2.22 (s, 3H, C12-CH$_3$), 1.84 and 1.80 (2s, 2 × 3H, C2-CH$_3$ and C18-CH$_3$), 1.22 and 1.20 (2t, 2 × 3H, CH$_2$C*H*$_3$); ^{19}F NMR (470 MHz, $CDCl_3$) δ −55.48 (s); UV−vis (CH_2Cl_2) λ_{max} [nm] (I_{rel}) 358 (3.75), 616 (1); FABMS [(+), 3-nitrobenzyl alcohol] *m*/*z* 669 (M + 1).

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Supporting Information Available: ^{19}F NMR spectra for the reaction mixture obtained upon coupled oxidation, hydrolysis, and esterification of porphyrin **2** and for compounds **4**, **5**, **6**, and **7**, ^{1}H NMR spectra for compounds, and ^{13}C NMR for compound **7** (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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