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Characterization of *meso*-rhodins and their Fe(III) Complexes

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Abstract

The *meso*-rhodin ester **1** and acid **2** as well as their corresponding ferric forms **3** and **4** have been characterized by UV, IR, ^{1}H NMR and FAB mass spectrometry. All these species are synthesized as equimolar mixtures of two isomers (6- or 7-propionic acid condensed into ring). Some separation of the isomers of **1** can be achieved by semipreparative HPLC. The 300 MHz ^{1}H NMR of the biscyano complexes of both **3** and **4** show almost all resonances for both isomers. A resonance for the α-CH_2 of the exocyclic ring is seen at >20 ppm. The FAB mass spectra indicated electron capture by the macrocyclic ring or Fe(III) atom.

Introduction

The heme prosthetic group is at the active site of many proteins. Although the heme performs many different functions (electron transfer, oxygen binding, decomposition of peroxides, oxidation of substrates), the structure of the heme is almost invariant. One aspect of this structure that is important in governing the reactivity of the heme is the presence of two propionic acid chains on one side of the heme tetrapyrrole macrocycle. It has been shown that these propionates are involved in binding one protein to another in a number of instances [1–6] and it has been postulated that ionization governs the redox potential of the heme [7, 8]. In a number of instances it has been observed that the activity of the protein is pH dependent, with a pK_a between 4 and 6 [1, 9–11]. In these cases, it has often been proposed that ionization of one of the heme propionates is responsible for the pH-dependent activity of the protein.

One way of investigating the role of the heme propionates in defining the biochemistry and spectroscopy of heme proteins is to study model compounds. Most model studies to date have utilized the natural hemins, which have two propionic acid chains. These two chains in natural hemins have very similar chemical characteristics and react simultaneously in both derivatization [12] and titration [13] experiments. It is therefore of use to have porphyrins which bear either only the 6-propionate or only the 7-propionate.

One approach to such a system is to use the *meso*-rhodin isomers shown below. These porphyrin derivatives were first synthesized by Fischer and co-workers in the 1930's [14–16], and were studied again by Fuhrhop and co-workers in the 1960's [17, 18]. The methyl esters of the free bases have been separated by fractional recrystallization [17, 18] and the lower melting isomer assigned as the isomer with the 6-propionic acid condensed onto the *meso* position [20]†. It has also been shown that the *meso*-rhodins can be converted back into the porphyrins [20]. Thus, synthesis of the *meso*-rhodins may provide a method for selective functionalization of either the 6- or the 7-propionate.

The *meso*-rhodin ring system is also of interest in conjunction with studies on petroporphyrins [21, 22]. Porphyrins derived from oil and oil shale have a number of interesting structures, often involving condensation of one of the side chains of the tetrapyrrole onto a *meso* position. The characterization of porphyrins derived from oil, the origin of these structures and the synthesis of new porphyrins

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†The structural assignment was based on oxidation of the *meso*-rhodins to the chloroporphyrin e_5 acids [14, 19]. The single isomer chloroporphyrin e_5 with only the 6-propionic acid chain attached to the *meso* position can be made from chlorophyll [14, 19]. Isomer assignments were made via a series of mixed melting points on various porphyrin derivatives [14]. In many instances it was not possible to separate the two isomers completely via fractional recrystallization. In these cases, the mixed melting points were taken of mixtures of the pure isomer (derived from chlorophyll) and various isomer mixtures derived from the porphyrin. The structural assignment must therefore be regarded as tentative.

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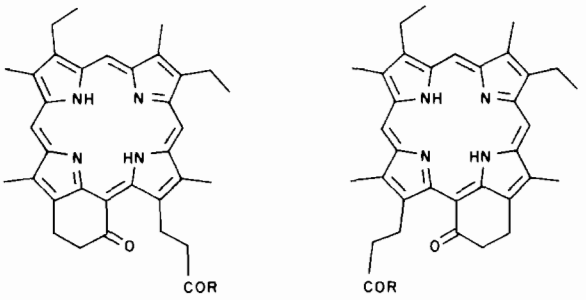
bearing exocyclic rings [23, 24] are all of current interest.

In this paper we report the characterization of *meso*-rhodin in the porphyrin methyl ester **1**, porphyrin acid **2**, Fe(III) ester **3** and Fe(III) acid **4** forms.



1, R = OMe
2, R = OH

Experimental

Materials

meso-Porphyrin IX dimethyl ester (Midcentury), fuming sulfuric acid (Fisher), and ferric chloride (Alfa) were all used as received. Pyridine (Omnisolv) was distilled from CaH_2. All deuterated solvents were from MSD isotopes. Silica gel-G plates (Analtech 1000 microns) were used for preparative TLC. Eastman chromatography sheets (Si gel) were used for analytical TLC. The HPLC instrumentation consisted of a Beckman 160 absorbance detector with 405 nm lamp, two model 110 pumps and a Whatman C-18 reverse phase column (M9 10/25 ODS-2). Solvent mixture and flowrate were controlled with an Axxiom 710 HPLC controller.

Spectral Measurements

Infrared spectra were taken on a Nicolet 7000 FT–IR instrument. UV–Vis spectra were recorded on a Cary 219 spectrophotometer. ^1H NMR spectra were recorded on Varian XL-300 and Bruker WH-360 spectrometers. All 2D experiments were done on the Varian. Spectra had 16 K data points with a spectral width of 6000 Hz for porphyrins and 12 000 Hz for Fe(III) porphyrins. Chemical shifts in ppm are referenced to the residual $CHCl_3$ or Me_2SO-d_5 signals assigned as 7.25 ppm and 2.50 ppm respectively. Fast atom bombardment (FAB) [25] mass spectra were recorded using a VG ZAB-SE double-focusing mass spectrometer equipped with a standard VG FAB source. Instrument conditions were: ion source 8 keV ion energy, FAB gun 8 keV Xenon atom beam with 1 mA gun emission current, instrument mass resolution 1100. Data were collected by scanning at a rate of 15 s/decade into a VG 11-250 data system, previously calibrated using the FAB spectrum of a mixture of cesium and rubidium iodides.

Synthesis

meso-Rhodin ester **1** and *meso*-rhodin acid **2** were prepared from *meso*-porphyrin IX dimethyl ester by an intramolecular Friedel–Crafts reaction following the procedure of Fischer *et al.* [14, 16]. *meso*-Porphyrin dimethyl ester (200 mg) was dissolved in 6 ml conc. sulfuric acid; 6 ml of fuming sulfuric acid was added carefully to avoid overheating. After ~1 h the burgundy color of the porphyrin had changed to green. The solution was then pipetted onto crushed ice with an excess of sodium acetate, neutralized with ammonium hydroxide, extracted with dichloromethane, washed with water and concentrated. The *meso*-rhodin ester **1** (dark green, faster running) and *meso*-rhodin acid **2** (dark green, slower running) were separated by preparative TLC (10% MeOH in $CHCl_3$).

meso-Rhodin methyl ester **1**: UV ($CHCl_3$; λ_{max}): 408, 511, 550, 580, 636 nm; (ether; λ_{max}, relative intensity): 401 (100), 504 (12.5), 545 (4.4), 580 (2.5), 635 (4.4) nm; literature [15] (end absorption at 437, 510.6, 545.5, 583.3, 636.6); IR (Me_2SO): 1733 (ester) and 1664 (conjugated carbonyl) cm^{-1}.

meso-Rhodin acid **2**: UV ($CHCl_3$; λ_{max}): 410, 513, 551, 584, 637 nm; (ether; λ_{max}, relative intensity): 406 (100), 508 (10.5), 545 (7.3), 585 (5.3), 635 (8.8) nm; literature [15] (end absorption at 437, 510.6, 545.4, 583.3, 636.6); IR (Me_2SO): 1716 (acid) and 1664 (conjugated carbonyl) cm^{-1}.

Iron was inserted into both **1** and **2** to convert them to **3** and **4** respectively following the procedure described by Chang *et al.* [26]. Purification was achieved by preparative TLC (eluted with 5% MeOH in $CHCl_3$).

Iron(III) *meso*-rhodin methyl ester **3**: UV (pyridine; λ_{max}, relative intensity): 416 (100), 568 (11) nm.

Iron(III) *meso*-rhodin acid **4**: UV (pyridine; λ_{max}, relative intensity): 418 (100), 568 (8).

Results and Discussion

Synthesis

The *meso*-rhodins were synthesized via an intramolecular Friedel–Crafts reaction as reported by Fischer and co-workers [14–16]. When a fresh bottle of fuming sulfuric acid was used, the reaction mixture turned from burgundy to bright green in approximately 1 h. TLC showed this mixture to have two major components, the *meso*-rhodin ester **1** and the *meso*-rhodin acid **2**. The reaction ran more slowly when old bottles of fuming sulfuric acid were used and the product mixture was more complex. TLC (20% MeOH in $CHCl_3$, silica gel) showed materials at the origin and 4 bands with R_f values of 0.86 (dark

green, *meso*-rhodin methyl ester), 0.42 (red, *meso*-porphyrin monoacid monoester), 0.21 (dark green, *meso*-rhodin acid), 0.13 (light green, unknown).

HPLC separation of the isomers of **1** on a semi-preparative scale proved difficult. Various mixtures of solvents (acetone, acetonitrile, ethyl acetate, dichloromethane, chloroform, methanol, water) were tried on both normal and reverse phase columns. The following system was the best of those tried: Whatman C-18 reverse phase column (M9 10/25 ODS-2): at $t = 0$ min, flow = 1.0 ml/min of 100% MeOH; at $t = 66$ min, flow = 1.0 ml/min of 3% H_2O in MeOH; at $t = 79$ min, flow = 3.0 ml/min of 3% H_2O in MeOH. Two fractions were collected at 95 min and 97 min. The ^1H NMR spectra of the two fractions show incomplete separation of the 6-, 7- isomers but some clarification of the multiplet regions.

^1H NMR

Resonance assignments for the ^1H NMR spectra of **1**–**4** are given in Table I. Assignments were made using resolution enhancement, 2D-J and 2D COSY techniques. For the free base rhodins, the spectra were complicated by the great similarity of the ^1H NMR resonances of the two isomers, which were not completely separated by HPLC.

In the *meso*-rhodin ester **1** the CH_3 of the 2- and 4-ethyl groups appeared as overlapping triplets at 1.80 and 1.84 ppm. The isomers were almost indistinguishable at 300 MHz. The CH_3 triplets were

TABLE I. ^1H NMR Spectra Data for *meso*-Rhodins and the Fe(III) *meso*-Rhodins[a]

Substituent	1[b]	2[c]	3[d]	4[d]
Ethyl[e]				
CH_3	1.80	1.84	−1.02	−0.93
CH_2	3.95	3.9–4.2	2.74	3.07
CH_3	1.84	1.84	−1.10	−0.93
CH_2	4.07	3.9–4.2	4.88	5.17
CH_3	f	f	−0.07	−0.13
CH_2	f	f	6.64	6.53
CH_3	f	f	−0.17	−0.30
CH_2	f	f	8.30	7.10
Propionic acid/ester chain				
αCH_2	4.19	3.9–4.2	5.20	7.27
βCH_2	3.23	3.22	−0.17	−0.93
αCH_2	f	f	5.33	7.43
βCH_2	f	f	−0.22	−0.93
Exocyclic ring				
αCH_2	4.19	3.9–4.2	25.3	23.76
βCH_2	4.00	3.9–4.2	1.18	0.87
αCH_2	f	f	f	f
βCH_2	f	f	f	f
meso	10.0–10.2	9.9–10.2	−1.36	−0.80
			g	−0.49
				−0.08
				g
Ring and ester methyls	3.5–3.7	3.5–3.7	3.10[h]	
			8.62	9.64
			8.76	10.64
			9.15	10.77
			13.26	13.52
			15.46	15.44
			18.54	16.47
			18.62	17.55
			18.68	17.66

[a]All compounds are equimolar mixtures of the 6- and 7-cyclized isomers. [b]In $CDCl_3$; 300 MHz. [c]In $CDCl_3$; 360 MHz. [d]In Me_2SO-d_6; 300 MHz; low-spin biscyano species. [e]Ethyl groups at both 2- and 4-positions. [f]Not resolved from resonances given above. [g]Other *meso* resonances not observed. [h]Ester methyl.

coupled to a six-proton multiplet at 3.9–4.1 ppm which was in turn coupled to a four-proton multiplet at 4.19 ppm. The multiplet at 4.19 ppm was coupled to a two proton multiplet at 3.23 ppm. This last multiplet is assigned as the 6,7-β-CH_2 of the propionic ester chain by analogy with *meso*-porphyrin dimethyl ester itself (β-CH_2 at 3.29 ppm). The four-proton multiplet at 4.19 ppm is therefore due to the α-CH_2 protons of both the propionic ester chain and rhodin ring. The NH resonances of **1** appeared upfield at −3.1 ppm. This position is indicative of a *meso*-substituted porphyrin. Porphyrins without *meso* substituents have their NH resonances at ~ −3.8 ppm [27]. The ^{1}H NMR of the *meso*-rhodin acid **2** was similar to that of **1** however the NH resonance of **2** was not observed, perhaps because it was very broad [27].

The ^{1}H NMR spectrum of the biscyano complex of the Fe(III) *meso*-rhodin ester **3** is shown in Fig. 1. All eight ring methyls of the two isomers are seen clearly as singlets between 8 and 19 ppm. The

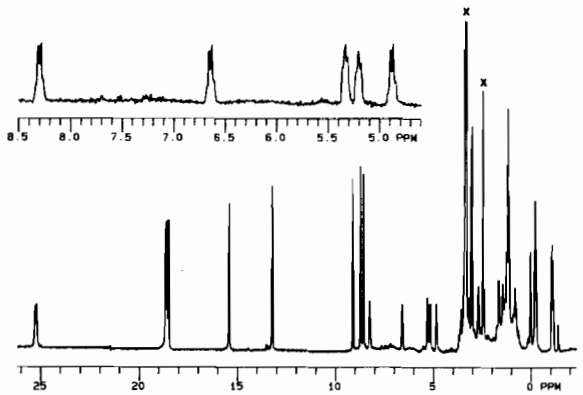


Fig. 1. Fe(III) *meso*-rhodin ester $(CN^-)_2$ in Me_2SO-d_6. Inset is the region between 4.5 and 8.5 ppm expanded with resolution enhancement to show the α-CH_2 resonances of the 2- and 4-ethyl groups and the propionic ester chain. Resonances marked with an X are due to HOD and Me_2SO-d_5.

furthest downfield resonance at 25.3 ppm integrates to four protons and is coupled to a multiplet at 1.18 ppm. These resonances are assigned respectively as the α- and β-CH_2 groups of the exocyclic ring. The α- and β-resonances of the free propionic ester chain are expected to be triplets. A resolution enhanced enlargement of the spectrum between 4.6 and 8.5 ppm shows two triplets and three quartets. By analogy with Fe(III) *meso*-porphyrin dimethyl ester $(CN^-)_2$, the triplets are assigned as the α-resonances of the propionic ester chains in the two isomers of **3**. The three quartets are three of the four ethyl α-CH_2 groups; the fourth α-CH_2 group is found at 2.74 ppm. The cross peaks in the 2D COSY spectrum allowed the assignments of the 2-, 4-, 6- and 7-β resonances in Table I. We have not assigned individual resonances to the two isomers of the mixture because we have been unable to separate enough **1** by HPLC to give clean **3** after iron insertion. We have recently shown that the nuclear Overhauser effect may be used to assign resonances of low-spin paramagnetic hemins [28]. However, to make the assignments in a 1-D spectrum all of the *meso* resonances must be resolved, and **3** shows only one resolved *meso* resonance.

The ^{1}H NMR of the Fe(III) *meso*-rhodin acid **4** was similar to that of **3** except that resolution enhancement did not allow assignment of all of the CH_2 groups as triplets or quartets. A partial assignment of the spectrum is given in Table I.

FAB Mass Spectrometry

In the positive ion spectra, the *meso*-rhodins **1** and **2** showed the expected $[M + H]^+$ peak as the largest peak in the cluster around the parent ion region (Table II). In the negative ion spectra the most intense peaks in the parent ion region were seen at an m/z of $M^{-\cdot}$. This is unexpected. The usual negative ion molecular signals are $[M-H]^-$ deprotonated molecules. However, the $[M-H]^-$ ions were smaller than the $M^{-\cdot}$ signals for these compounds, being approximately 40% and 67% of the $M^{-\cdot}$ peak for the

TABLE II. Positive and Negative Ion FAB Mass Spectra of *meso*-Rhodins and Fe(III) *meso*-Rhodins

Compound	Mass spectra	Relative intensity of ions[a]					
		$M-2$	$M-1$	M	$M+1$	$M+2$	$M+3$
1	positive	15	52	100	370	174	70
$M = 562$	negative	22	40	100	81	44	24
2	positive	13	55	100	250	200	110
$M = 548$	negative	33	67	100	84	35	15
3	positive	23	57	100	73	28	10
$M = 616$	negative	91	88	100	59	19	10
4	positive	21	54	100	64	25	9
$M = 602$	negative	69	99	100	52	16	6

[a]Run to run difference ±10.

ester **1** and the acid **2** respectively. Thus, electron capture is favored over deprotonation as the dominant process in negative ion formation, a situation not observed previously in FAB–MS of organic molecules. We suggest that electron capture is a competitive process with deprotonation for negative ion formation. For a majority of organic molecules the latter process is favored because a suitably low-energy unoccupied molecular orbital able to accommodate the extra electron is absent. However, molecules such as *meso*-rhodins in which a substantial aromatic system exists have low-energy lowest unoccupied molecular orbitals (LUMOs) which enable electron capture processes to compete with, or dominate, the deprotonation mechanisms. This is analogous to the mechanisms for positive ion formation in FAB–MS discussed by Cerny and Gross [29].

Both the iron-containing complexes (**3** and **4**) showed $M^{+\cdot}$ signals in the positive ion FAB mass spectra. However, their associated isotope patterns appearing at ($[M + H]^+$ etc.) were generally larger than expected based upon $M^{+\cdot}$ being the only molecular species present. There are two possible explanations for this behavior. First, the ions containing Fe(III) might have added hydrogen radicals; similar adducts have been observed for certain organic molecules such as flavin mononucleotide [30]. An alternative explanation is that a certain amount of material is reduced to the Fe(II) complex (either prior to analysis or in the FAB process). This neutral species would be ionized by proton addition and seen as the conventional $[M + H]^+$ ion which appears one mass unit higher than the $M^{+\cdot}$ ion of the Fe(III) species. It is not possible to differentiate these processes, although the former is also observed for non-metal-containing organics which contain a conjugated system with one or more heteroatoms and therefore a precedent exists for this rationalization. The negative ion spectra showed a mixture of the M^- and $[M-H]^-$ species of the Fe(II) complex. Negative ion adducts of the Fe(III) complex (such as $[M + H]^-$) were observed with minor intensity.

Conclusion

The *meso*-rhodin acid and esters have been synthesized and iron inserted. Even at 300 MHz, the two isomers of the free base rhodins have very similar spectra. However, the difference between the two isomers is very clearly seen in the spectra of the Fe(III) rhodin $(CN^-)_2$ complexes. Mixtures of the isomers can therefore be characterized relatively rapidly in their ferric forms. The FAB mass spectra of the rhodins are most easily explained by invoking electron capture by the macrocycle or Fe(III) center.

Acknowledgments

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