The hydroxylation and amidation of equilenin acetate catalyzed by chloro[5,10,15,20-tetrakis(pentafluorophenyl)porphyrinato]manganese(III)

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The aromatic steroid equilenin acetate undergoes regioselective and stereoselective hydroxylation and amidation catalyzed by a manganese porphyrin using iodosobenzene (PhIO) and *N*-tosyliminophenyliodinane (PhINTs) as the oxygen and nitrogen donor, respectively.

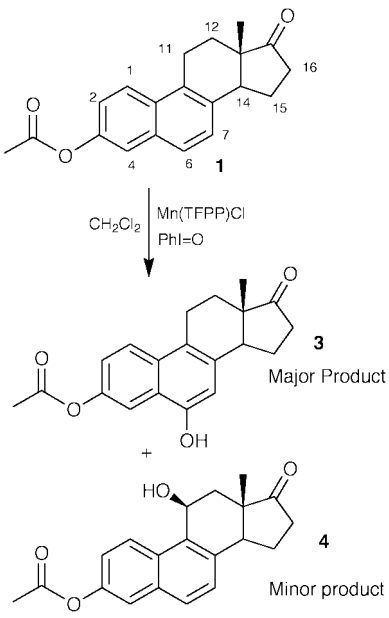
It is important to develop chemical methods for the regiospecific oxidation of natural products such as steroids to replace microbial fermentations that are currently used.[1] Biochemically, such oxidations are normally performed by the heme-containing cytochrome P-450 class of enzymes, and metalloporphyrins have been studied as models for these enzymes.[2] We have recently reported the regiospecific catalytic hydroxylation of steroids, using a water soluble porphyrin carrying hydrophobic binding units, that performs efficient and specific oxidations directed by the well defined geometry between the catalyst and substrates.[3–5] Herein, we report that the aromatic steroid equilenin acetate **1** can be hydroxylated and amidated at very specific positions with good catalytic turnover by iodosobenzene (PhI=O) and *N*-tosyliminophenyliodinane (PhI = NTs),[6] catalyzed by chloro[5,10,15,20-tetrakis(pentafluorophenyl)porphyrinato]manganese (III) **2**.

The metalloporphyrin-catalyzed oxidation of substituted arenes with several oxidants has been studied by the groups of Baciocchi[7] and Meunier.[8] Both groups found that quinones were the dominant products produced from these reactions, especially in the case of substituted naphthalenes. However, when we treated a 54 mM solution of equilenin acetate **1** with 270 mM PhI=O in the presence of 54 mM chloro[5,10,15,20-tetrakis(pentafluorophenyl)porphyrinato] manganese(III) **2**, no quinones were produced after 12 h. Instead, the major product isolated was the 6-hydroxylated product **3**, along with a smaller amount of the 11β-OH product **4** (Scheme 1). No starting material was recovered. Analytical HPLC studies using a catalyst:substrate:oxidant mole ratio of 1:50:250 (CH_2Cl_2, 23 °C, 12 h) resulted in a 57% conversion of **1** to 42% of **3** and 15% of **4**, indicating *ca*. 25 catalytic turnovers (Scheme 1).†

The products were characterized by MS and NMR spectra. Compound **3** had a ^{1}H NMR spectrum with one less aromatic proton than the starting material. COSY spectroscopy confirmed coupling between the 1, 2, and 4 protons as well as the allylic coupling between the 7 and 14 protons, indicating that hydroxylation occurred at C-6.

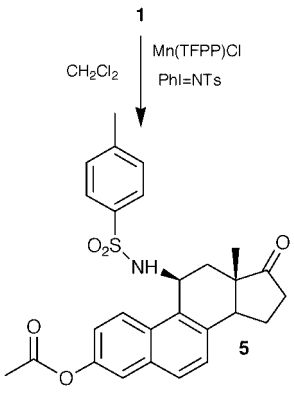
Compound **4** lacked the original C-11 benzylic methylene group signal at 3.3–3.4 ppm, and had a new peak at 5.77 ppm as expected for a downshifted C-11. The C-11 proton showed coupling to a methylene group with no other neighbors (C-12), and also showed NOE coupling to the C-1 proton. This coupling indicates that the H on C-11 is equatorial, so the OH is on the β face. This 11-β OH assignment is also consistent with an upfield shift of the angular methyl group from 0.80 ppm in **1** to 0.72 ppm in **4**. The remaining C-14, C-15, C-16 coupled protons and the aromatic protons were still present.

We first reported the metalloporphyrin catalyzed amidation of organic compounds in 1982.[9] Most recently, Che and coworkers reported the asymmetric amidation of substituted naphthalenes using chiral ruthenium and manganese porphyrins.[10] We find that such an amidation can also be performed on the equilenin steroid substrate **1** with good selectivity. A 60 mM solution of **1** was stirred for 12 h under argon in 1 ml of distilled CH_2Cl_2 containing metalloporphyrin **2** (60 mM), PhI=NTs (300 mM) and molecular sieves to produce the 11β-amidation product **5** and trace amounts of **3** and **4**, with complete conversion of starting material (Scheme 2).† In contrast to hydroxylation, the amidation reaction went completely to the 11β position of the steroid without any detectable amidation at the 6 position. A mole ratio of 1:50:250 catalyst:substrate:PhI=NTs afforded an 82% conversion of **1** to 30% **3**, 5% **4** and 47% **5**, as determined by HPLC assay, so there are *ca*. 40 turnovers.



Scheme 1

Compound **5** had the expected MS, and again a downfield shifted C-11 proton at 5.43 ppm was coupled to the C-12 methylene and showed NOE coupling to the C-1 proton. Thus the tosylamide group is attached at the 11-β position. The



Scheme 2

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angular methyl group shifts strongly upfield from 0.80 ppm in **1** to 0.55 ppm in **5**.

The hydroxylation products **3** and **4** reflect hydrolysis of the Mn=NTs intermediate by traces of water in the small scale analytical runs, and they are minimally present in larger preparative scales. In previous work, we had found that the enzyme cytochrome P-450 could aminate cyclohexane with PhI=NTs, but that some hydroxylation also occurred in the water solution.[11] Since the relative amount of hydroxylation depended on the particular isoform of the enzyme used, hydrolysis of the metalloporphyrin intermediate was the most likely explanation. The finding that benzylic substitution in compounds **4** and **5** occurs on the beta face of the steroid must reflect the stereoelectronic control of the flat conjugated benzylic radical intermediate in these reactions.

The readily available manganese porphyrin **2** is able to catalyze the hydroxylation and amidation of an aromatic steroid. These reactions are especially interesting for their apparent differences in regiospecific oxidations. Although not explicitly stated in the literature, metalloporphyrin catalyzed oxidations of substituted naphthalene compounds have been reported to afford mostly quinones after aromatic ring hydroxylation in the presence of oxygen donors, whereas they tend to produce only amides at previously saturated carbon positions in the presence of PhINTs as the nitrogen donor.[7–10]

Apparently the oxidations involve preferential oxygen atom donation to the aromatic ring before hydrogen loss, while the tosylamidations involve initial hydrogen removal from a benzylic position. Perhaps aromatic ring addition is more sterically demanding, and thus more available to a small oxygen atom than to a large tosylamide group. We see that this difference generally holds true in the case of equilenin acetate and provides a method by which steroids of this class can be functionalized at different positions. Furthermore, the amidation of a steroid substrate can lead to the development of a novel class of nitrogen containing steroids that may have useful biological properties.

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Notes and references

† Equilenin acetate was synthesized by acylation of equilenin (Steraloids, Inc.) with acetic anhydride in pyridine using standard procedures. All products were isolated by column chromatography and characterized by ^{1}H-NMR, COSY, NOESY and CI-MS.

3: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.86 (1H,d, C4-H), 7.82 (1H, d, C1-H), 7.41 (1H, dd, C2-H), 6.29 (1H, d, C7-H), 3.38 (1H, ddd, C14-H), 2.34 (3H, s, acetate Me), 0.76 (3H, s, C18-Me), 2.8–1.9 (8H, steroid envelope). CI-MS: m/z = 342 (M + 1 + NH_3), 323 (negative, M − 1). Product **3** in its ^{1}H NMR spectrum showed one less aromatic proton as compared to the starting material. COSY spectroscopy confirmed coupling between the 1, 2 and 4 protons as well as the allylic coupling between the 7 and 14 protons. All remaining aliphatic protons were identified by COSY, also confirming the identity of the product as C6-hydroxylated equilenin acetate.

4: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.38 (1H, d, C1-H), 7.83 (1H, d, C6-H), 7.60 (1H, d, C4-H), 7.37–7.28 (2H, m, C7-H and C2-H), 5.77 (1H, br m, C11α-H), 3.43 (1H, m, C14-H), 2.39 (3H, s, acetate Me), 0.72 (3H, s, C18-Me), 2.83–1.91 (6H, steroid envelope). CI-MS: m/z 342 (M + 1 + NH_3). Product **4** COSY indicated coupling of both C15 hydrogens to the easily identifiable C14 proton at 3.43 ppm. The C15 protons were coupled to the C16 protons, indicating that oxidation must have occurred on the C11 or C12 steroid position. The C11 protons, originally at 3.3-3.4 ppm in **2**, were not present in product **4** and a new C*H*–OH appeared at 5.7 ppm, consistent with a benzylic oxidation. The relatively large upfield shift of the angular C18 methyl is inconsistent with C12 oxidation and is furthermore consistent only with oxidation occurring on the beta face of the last remaining steroid carbon position at C11 (most C11 α hydroxlations occur with large downfield shifts of the C18 methyl). Lastly, strong NOE coupling between the C1-H and the C11α-H indicated that compound **4** was indeed the 11β hydroxylated product.

In the analytical runs, the formation of products relative to starting material were monitored with 2-methoxynaphthalene as an internal standard, comparing NMR ratios and HPLC responses to calibrate the relative absorption coefficients of the products and starting material in the UV-VIS HPLC detector.

5: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.81 (1H, d, C1-H), 7.77 (1H, dd, C6-H), 7.72 (2H, d, toluenesulfonamide), 7.52 (1H, d, C4-H), 7.31 (1H, dd, C7-H), 7.29(2H, d, toluenesulfonamide), 7.09 (1H, dd, C2-H), 5.43 (1H, ddd, C11α-H), 4.45 (1H, d, N–H), 3.36 (1H, m, C14-H), 2.45 (3H, s, toluene Me), 2.36 (3H, s, acetate Me), 0.55 (3H, s, C18-Me), 2.66–1.96 (6H, steroid envelope). CI-MS: m/z 495 (M + 1 + NH_3). The same COSY pattern was present as with product **4**. The C14 hydrogen was coupled to both C15 hydrogens, which in turn were coupled to both C16 hydrogens indicating oxidation at C11 or C12. Again, there was a large upfield shift of the angular C18 methyl, and the new CH-NH at 5.43 ppm is consistent with a benzylic oxidation. Also, strong NOE coupling between the C1-H and the C11α-H confirmed **5** as the 11β amidated product.

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