Regio- and stereo-selective oxidation of steroids using 2,6-dichloropyridine *N*-oxide catalysed by ruthenium porphyrins

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Ruthenium porphyrins catalyse oxygen transfer from 2,6-dichloropyridine *N*-oxide to steroids with retention of configuration at the asymmetric centre, giving novel steroids.

Various oxidation systems for steroids have been developed,[1] and recently there has been increasing interest in metalloporphyrin-catalysed oxidation systems as a model of cytochrome P450 for alkanes[2] and steroids.[3] We have already shown that ruthenium porphyrins effectively catalyse the oxidation of alkenes, sulfides and alcohols in the presence of heteroaromatic *N*-oxides.[4] In particular, this catalytic system efficiently oxidizes unactivated aliphatic or aromatic hydrocarbons in high yield with an extremely high turnover number (up to 10^5 times) in the presence of HCl or HBr. Here we report the regio- and stereo-selectivity of oxidation in steroids using this system.[5]

We investigated the oxidation of 5α-cholestan-3-one **1** with 2,6-dichloropyridine *N*-oxide catalysed by Ru(TPP)CO, Ru(TMP)CO and Ru(TDClPP)CO (Fig. 1) in the presence of HBr (runs 1–3). In the reaction catalysed by Ru(TPP)CO, **1** was oxidized to afford the 25-hydroxy derivative **2** in 11% yield (run 1). When the reaction was catalysed by Ru(TMP)CO or Ru(TDClPP)CO, **1** was oxidized to afford (20*S*)-20,25-dihydroxycholestan-3-one **3**, (20*S*)-20-hydroxycholestan-3-one **4** and 25-hydroxycholestane-3,15-dione **5** in addition to **2** (runs 2 and 3). The structures of these novel steroids **3**–**5** were identified by mass spectroscopy, ^{1}H and ^{13}C NMR analysis and IR analysis. The mass number of **3** was larger than that of the starting material **1** by 32 *m/z*. In the ^{13}C NMR spectrum, methine carbon signals (δ 36, 28) were replaced by quaternary carbons (δ 74, 71, respectively), and in the ^{1}H NMR spectrum, the signals of the methyl protons at the 21-, 25- and 26-positions each appeared as a singlet. The configuration of **3** at the 20-position was determined by an X-ray crystal structure analysis (Fig. 2).‡ Thus, hydroxylation with our catalytic system proceeds with retention of the configuration of the asymmetric centres. It is noteworthy that in the biosynthesis of progesterone, cholesterol is hydroxylated with retention of configuration at the 20-position *in vivo* by P450.[6] At least in this respect, our system can be regarded as a model of P450.

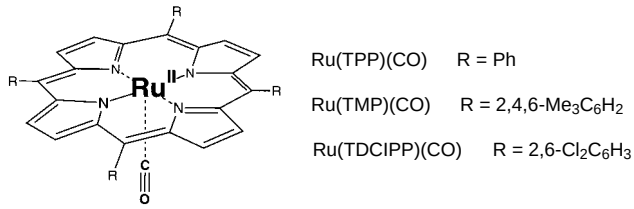


Fig. 1 Structure of ruthenium porphyrins

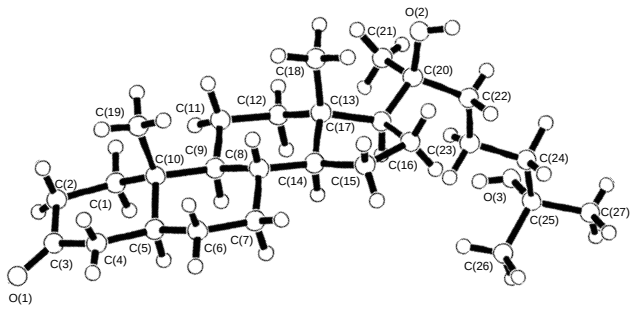
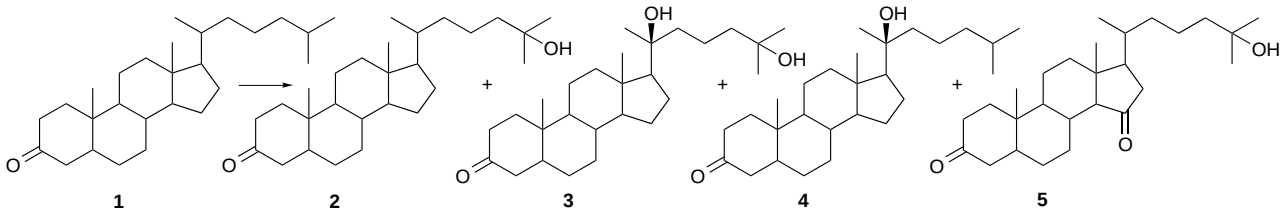


Fig. 2 Molecular structure of (20*S*)-20,25-dihydroxycholestan-3-one **3**

The position of the hydroxy function of **4** and **5** was similarly identified. The location of the carbonyl function of **5** was supported by 2D NMR experiments (^{1}H-^{1}H and ^{13}C-^{1}H shift correlation). From the results shown in Table 1 it can be suggested that the regioselectivity in the oxidation of **1** depends markedly upon the structure of the porphyrin. Because ruthenium porphyrins which have *o*-substituents on the aromatic ring at the *meso*-position showed higher reactivity than those which had no substituent (runs 2 and 3 *vs*. run 1), the regioselectivity may be influenced by changes in reactivity caused by steric hindrance arising from the *o*-substituents on the aromatic ring at the *meso*-position. Furthermore, Ru(TMP)CO has a different regioselectivity from Ru(TDClPP)CO, although the methyl group is as bulky as the chloride group (runs 2 and 3). This implies that variation of electron density caused by electron-donating or -withdrawing substituents on the aromatic ring at the *meso*-position is also important. Therefore, it can be assumed that the regioselectivity depends upon both the steric and electronic effects of the substituents.

Several other 5α-steroids were treated with this system (Scheme 2). As shown in Table 1, 5α-androstan-3,17-dione **6a** was not oxidized under these conditions and was recovered in 91% yield (run 4). 5α-Pregnan-3,20-dione **6b** was oxidized to afford the 5α- and 14α-hydroxy derivatives, **7b** and **8b**, respectively, in 18 and 6% yields, respectively (run 5). 5α-Cholestane **6c** was also oxidized in 73% yield and the 5α-hydroxy derivative **7c** was isolated in 28% yield (run 6). It

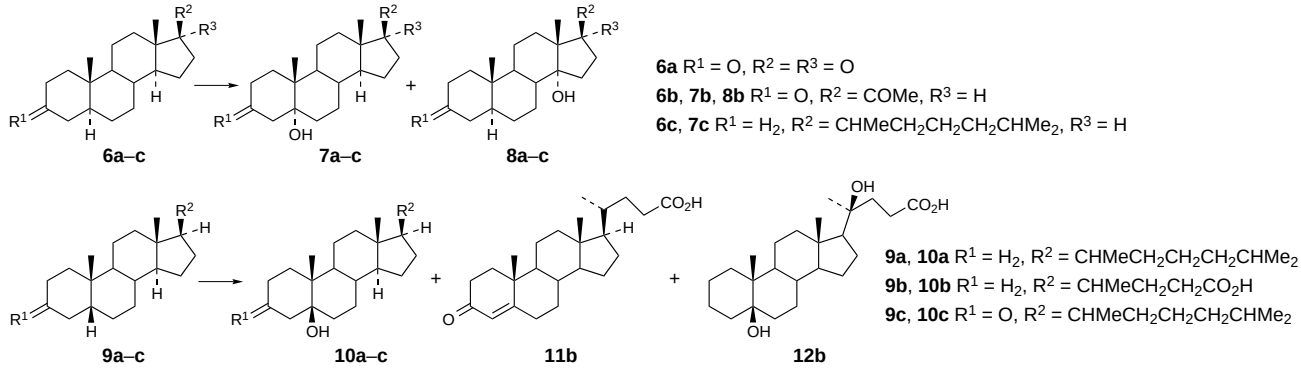


Scheme 1 Oxidation of 5α-cholestan-3-one **1** with the ruthenium porphyrin-2,6-dichloropyridine *N*-oxide system

Table 1 Oxidation of steroids with the ruthenium porphyrin-2,6-dichloropyridine *N*-oxide system[a]

Run	Substrate	Catalyst	Products (yield %)			
1[b]	**1**	Ru(TPP)CO	**2** (11%)			
2	**1**	Ru(TDClPP)CO	**2** (25%)	**3** (19%)	**4** (6%)	
3[c]	**1**	Ru(TMP)CO	**2** (26%)		**4** (7%)	**5** (12%)
4	**6a**	Ru(TMP)CO	None			
5	**6b**	Ru(TMP)CO	**7b** (18%)	**8b** (4%)		
6	**6c**	Ru(TMP)CO	**7c** (28%)			
7	**6c**	Ru(TMP)CO	**7c** (14%)[d]			
8	**9a**	Ru(TMP)CO	**10c** (64%)[d]			
9	**9b**	Ru(TMP)CO	**10b** (70%)	**11b** (8%)	**12b** (4%)	
10	**9c**	Ru(TMP)CO	**10c** (42%)			

[a] Reaction conditions: substrate (1 mmol), Ru(porphyrin) (2 μmol), 2,6-dichloropyridine *N*-oxide (3 mmol), 47% HBr (10 μl); molecular sieves 4 Å (150 mg) and benzene (6 ml) at 40 °C under an Ar atmosphere overnight. Yields are based on the substrate used. [b] 0.5 μmol of Ru(TPP)CO was used. [c] 4.5 mmol of 2,6-dichloropyridine *N*-oxide was used. [d] 1.2 mmol of 2,6-dichloropyridine *N*-oxide was used. The yields of **7c** and **10a** were determined by GLC.



Scheme 2 Oxidation of 5α- and 5β-steroids with the ruthenium porphyrin-2,6-dichloropyridine *N*-oxide system

appears that substrates are oxidized at tertiary carbons in this system.

On the other hand, 5β-steroids were oxidized to the corresponding 5β-hydroxy derivatives in a good yield (Scheme 2). 5β-Cholestane **9a** afforded **10a** in 64% yield (run 8). In contrast, 5α-cholestane **6c** under the same conditions afforded **7c** in 14% yield (run 7). Both **6c** and **9a** were hydroxylated with retention of configuration. It is assumed that this difference in reactivity at the 5-position is due to the difference in steric hindrance of hydrogen at the 5-position. 5β-Cholan-24-oic acid **9b** afforded **10b**, **11b** and **12b**, and **10b** was obtained with the best yield in this report (70%). Since **10b** was oxidized to **11b** with this system, it is assumed that **10b** was an intermediate in the formation of **11b**. The identification of **12b** as a novel steroid was based on ^{1}H and ^{13}C NMR spectra, mass spectra and IR analysis. Compared with **9a** and **9b**, 5β-cholestan-3-one **9c** was less reactive (run 10), so the carbonyl function appears to reduce the reactivity in its vicinity in this oxidation system.

Oxidation of estrogens was also examined. 3-Hydroxyestra-1,3,5(10)-trien-17-one (estrone: **13**) was converted to a mixture of oxidized products which could not be separated. However, estrone methyl ether **14** afforded the 6-keto derivative **15** in 21% yield.

We have shown that the ruthenium porphyrin–2,6-dichloropyridine *N*-oxide system oxidizes steroids with retention of the configuration of the asymmetric centres. This is a critical feature for the synthesis of optically active compounds, so this system could have synthetic applications. It showed moderate regioselectivity in the oxidation of steroids. Since hydroxylation at the 20 and 25 positions is observed when cholesterol and vitamin D_3 are metabolized to progesterone[6] and 25-hydroxy vitamin D_3[7] *in vivo* by P450, respectively, our system could be useful for research on steroid metabolites. Moreover, four novel steroids were obtained with our oxidizing system. Because the regioselectivity depended markedly upon the structure of the porphyrin, further modification of the porphyrin may afford new oxidized steroids, which could provide clues to novel metabolic pathways *in vivo*.

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Footnotes

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‡ *Crystal data* for (20*S*)-20,25-dihydroxycholestan-3-one **3**. $C_{27}H_{46}O_3$, M = 418.66, crystal dimensions (mm) 0.5 × 0.3 × 0.03, monoclinic, space group $P2_1$ (no. 4), a = 9.889(1), b = 7.5383(6), c = 16.7188(9) Å, β = 101.749(6)°, Z = 2, U = 1220.2(2) Å, D_c = 1.139 g cm^{-3}, μ = 5.53 cm^{-1}, Cu-Kα radiation, λ = 1.54178 Å, F(000) = 446.00, T = 293 K, R = 0.042, Rw = 0.035 [$w = 1/\sigma^2(F_o)$] for 1654 independent reflections [$I > 3\sigma(I)$] with 442 variables. The structure was solved by direct methods using MITHRIL90 and refined by full-matrix least-squares techniques using the TEXSAN program package of Molecular Structure Corporation. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See Information for Authors, Issue No. 1. Any request to the CCDC for this material should quote the full literature citation and reference number 182/405.

References

1 D. H. R. Barton, J. Boivin and P. Lelandais, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1797; Y. Mazur, *Pure Appl. Chem.*, 1975, **41**, 145; Z. Cohen and Y. Mazur, *J. Org. Chem.*, 1979, **44**, 2318.
2 In *Cytochrome P450: Structure, Mechanism and Biochemistry*, ed. P. R. Oritz de Montellano, Plenum Press, New York, 1986; B. Meunier, *Chem. Rev.*, 1992, **92**, 1411.
3 J. T. Groves and R. Neumann, *J. Org. Chem.*, 1987, **53**, 3891; P. A. Grieco and T. L. Stuk, *J. Am. Chem. Soc.*, 1990, **112**, 7799.
4 T. Higuchi, H. Ohtake and M. Hirobe, *Tetrahedron Lett.*, 1989, **30**, 6545; 1991, **32**, 7435.
5 H. Ohtake, T. Higuchi and M. Hirobe, *J. Am. Chem. Soc.*, 1992, **114**, 10 660; T. Higuchi, C. Satake and M. Hirobe, 1995, **117**, 8879; H. Ohtake, T. Higuchi and M. Hirobe, *Heterocycles*, 1995, **40**, 867; T. Higuchi, *J. Synth. Org. Chem. Jpn.*, 1995, **53**, 644.
6 M. Morisaki, S. Sato and N. Ikekawa, *FEBS Lett.*, 1976, **72**, 337.
7 J. W. Blunt, H. F. DeLuca and H. K. Schonoes, *Biochemistry*, 1968, **7**, 3317.

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