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Behaviour of Terpene Peroxides from Abietic Acid in the Presence of Heme and Non-Heme Iron(II)

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Abstract—In aqueous acetonitrile with $FeCl_2$ or hemin/L-cysteine, a peroxide (**4**) obtained from abietic acid by photosensitized air oxidation undergoes rearrangement to give a diepoxide and two spiro compounds as major products.

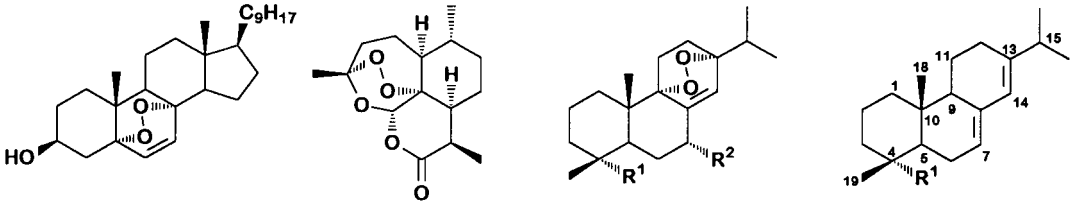
Introduction

Many naturally occurring endoperoxides provide pharmacological activity. Examples include ergosterol peroxide **1**, which has been obtained, e.g. from a variety of fungi and sponges,[1–3] and the sesquiterpene endoperoxide artemisinin (qinghaosu, QHS, **2**), isolated from *Artemisia annua*. While ergosterol peroxide shows antitumor[4] as well as antiviral[5] activity and has been reported to act as an immunsuppressive agent,[6,7] artemisinin has become a lead compound in the search for new fast acting antimalarials.[8]

The peroxy group is essential for antimalarial activity. The mode of action of QHS and its derivates has been suggested[9–12] to be in the intraerythrocytic stage, involving the cleavage of the peroxide bridge by heme iron[13] yielding a carbon centred free radical[14] which in turn alkylates parasite-specific proteins.[15] The reduction of hemin iron(III) to heme iron(II) is induced by an exogenous, possibly thiol based electron source.[16]

Several research groups have conducted investigations on the behaviour of QHS[17] against heme and non-heme iron since 1990. Some used QHS and closely related compounds, while others worked on very simple 1,2,4-trioxane systems.[18–31] It was suggested that other naturally occurring endoperoxides like **3** should also fulfil the mechanistic criteria for Fe^{2+}-promoted rearrangement and hence display antimalarial activity.[11]

Therefore we tested the reactivity of terpene peroxides (**4** and **5**) versus heme and non-heme iron(II) under different conditions. The cleavage reactions were carried out at room temperature under argon atmosphere in acetonitrile containing 1 equiv. of $FeCl_2 \cdot 4H_2O$ or aqueous acetonitrile (CH_3CN/phosphate buffer 1:1, pH 7.8) with 0.1–1 equiv. of hemin



1 **2**

3 R^1=COOH, R^2=H
4 R^1=$COOCH_3$, R^2=OH
5 R^1=CH_2OH, R^2=OH

6 R^1=$COOCH_3$
7 R^1=CH_2OH

Figure 1.

Keywords: terpenes; peroxides; radicals and radical reactions; rearrangements.
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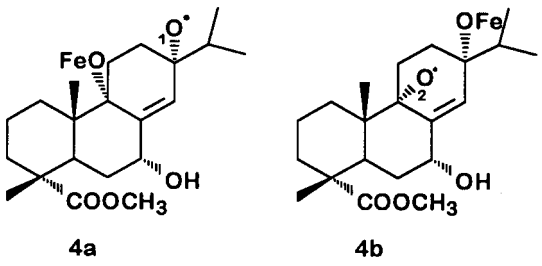


Figure 2.

and additional 1 equiv. of L-cysteine as electron donor. In this systems peroxides **4** and **5** could easily be cleaved. We used $FeCl_2 \cdot 4H_2O$ because it was reported to be the most active ferric salt in artemisinin cleavage reactions.[31] Like QHS,[31] **4** and **5** were completely decomposed at room temperature within a few minutes (Fig. 1).

The structures were determined using one- and two dimensional NMR techniques, specially ^1H, ^{13}C, DEPT, gCOSY, gTOCSY and $^{13}C,^1H$-correlations like gHSQC- and gHMBC-experiments. The relative configuration of chiral centres was established by NOESY experiments.

Results and Discussion

Peroxides **4** and **5** were obtained by photooxygenation[32–34] from methyl abietate **6**[35] or abietinol **7**. In the present work, ester **6** (alcohol **7** resp.) was dissolved in 96% ethanol containing methylene blue as sensitising dye. The solution was irradiated with light and sparged with oxygen simultaneously. Instead of a 60 W lamp[35] a high pressure mercury lamp was used with a 1% K_2CrO_4 solution as filter. Other parameters were optimised too. The yield reached 20–25% after purification by column chromatography. Peroxide **5** was obtained as white crystals with a mp of 195°C and could also be synthesised by reducing **4** with $LiAlH_4$. The behaviour of **4** and **5** is very similar to artemisinin. In a first step **4** forms two oxygen radicals (**4a** and **4b**, Fig. 2), which undergo rearrangement to form more stable carbon centered radicals. The subsequent transformations result in the formation of the products shown in Fig. 3.

In order to mimic physiological conditions, all cleavage reactions were performed in aqueous media. Exposure of **4** to 1 equiv. of $FeCl_2 \cdot 4H_2O$ in MeCN with stirring for at least 30 min at room temperature brought about complete reaction. In all cases **8** and **9** were the main products. **8** Was also found by Enoki and coworkers[36] when a solution of methyl abietate in *n*-hexane was exposed to sky light with aeration.

When the reaction was carried out in $MeCN/D_2O$, the C-3 deuterated product **14** could be isolated indicating an addition of a deuterium-radical from the solvent (loss of the coupling of Me 9 to 3-H in the ^1H-spectrum is observed).

As aforementioned, **4** and **5** were also treated with hemin in aqueous buffer. For a complete reaction it was not necessary to use a molar amount of hemin, although this was reported by Haynes and Vonviller for artemisinin cleavage reactions.[37,38] Based on our results—with 0.1 equiv. of hemin peroxide, **4** was completely decomposed within 21 h—we suggest a regeneration of Fe(II). We propose, that L-cysteine reduces first hemin-iron(III) to heme-iron(II), which donates one electron to the peroxide-bridge resulting in the cleavage of the O–O bond. According to Wu and co-workers[23] Fe^{2+} can be generated during the subsequent transformations of oxyl-radicals (like **4a** and **4b**), in our case this must be, for example, during the formation of substance **8**, as shown in Scheme 1. Products resulting from thiol transfer to **4** in the presence of hemin were not found. This corresponds to the results described by Haynes and Vonviller when QHS was treated with hemin in the presence of L-cysteine.[37,38]

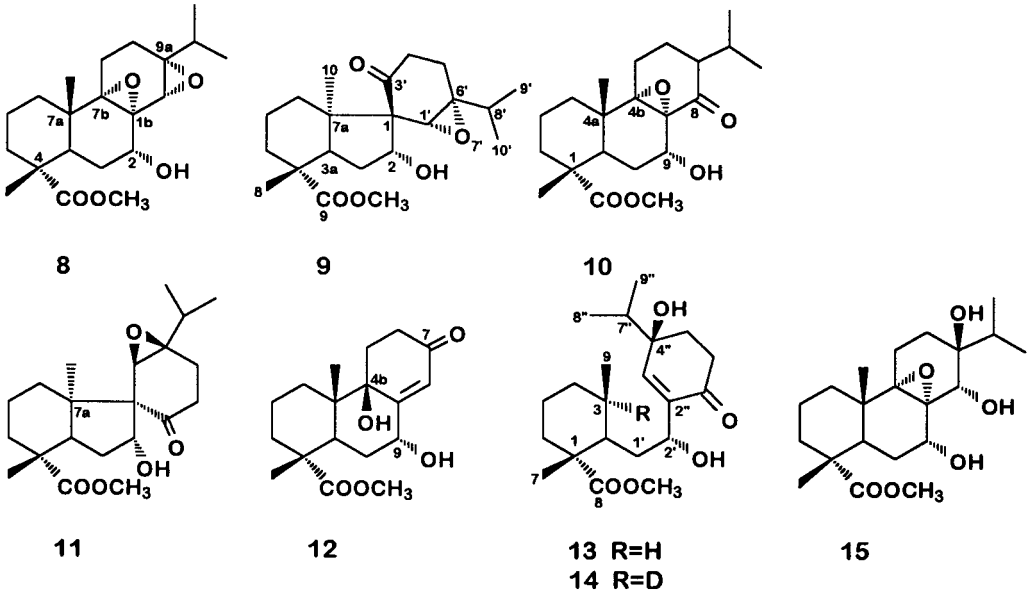
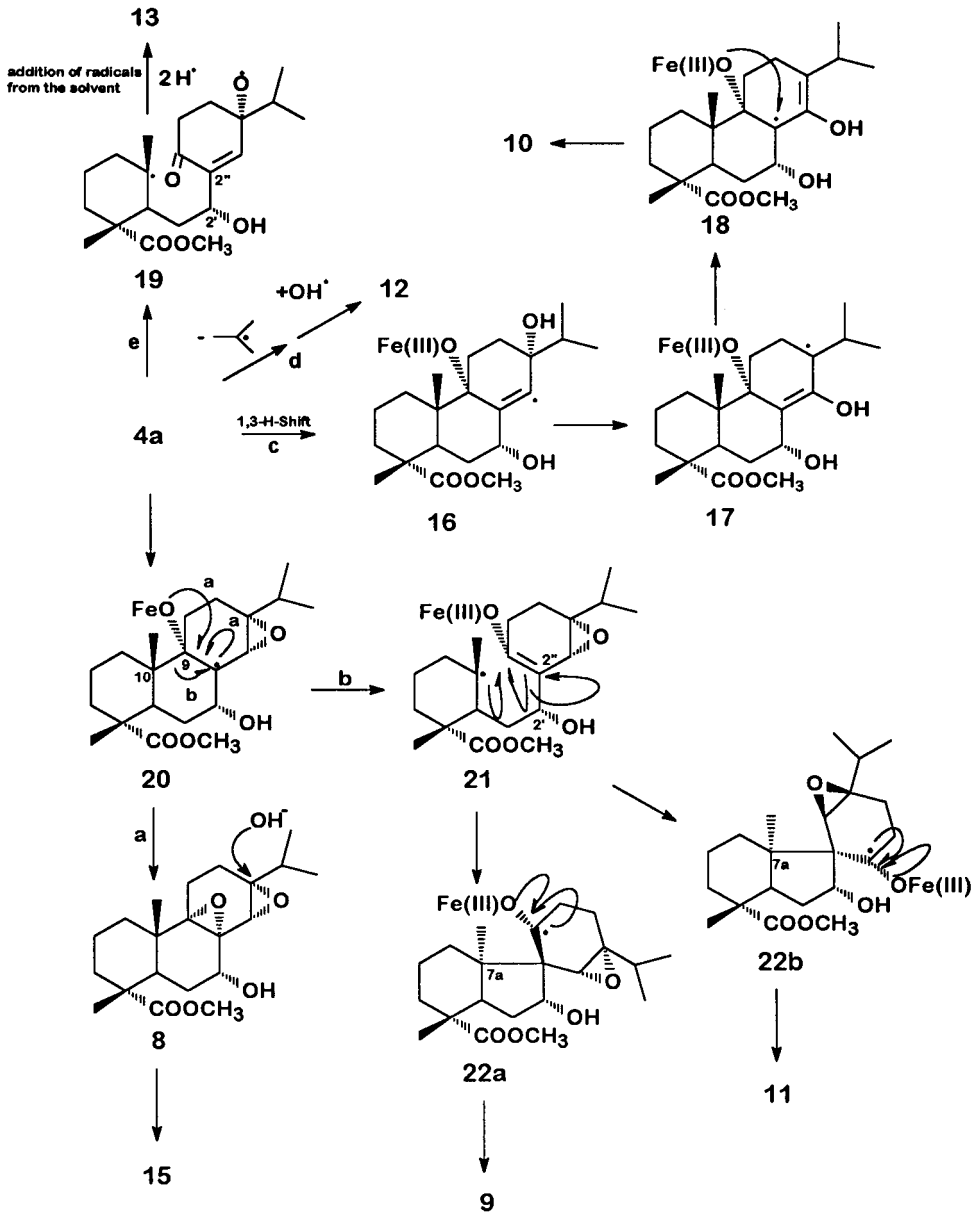


Figure 3.



Scheme 1. (a)–(e) Possible rearrangement pathways after the interaction of **4** with iron (for explanation see text).

4a And **4b** can undergo rearrangement in different ways as shown for **4a** in Scheme 1. The tertiary radical **20**, which is generated in a first step from **4a**, may have several pathways for further reactions (a–e). **8** Is formed by two intramolecular radical combinations (a). Attack of water to one of the oxirane rings of **8** yields **15**. **20** Can also rearrange (b) into radical **21** (scission between C-9 and C-10), which in turn forms the two radicals **22a** and **22b**. As a consequence of free rotation around the C-2′-C-2″ bond the two spiro compounds **9** and **11** are formed which differ only in the configuration of the spiro carbon. This has been proved by the NOE experiments: In difference to **11** irradiation of 3a-H in **9** gave an enhancement of the resonance of 7′-H. An NOE from 3a-H to CH_3 at C-7a indicates inversion of the configuration of this carbon in **9** and **11** compared to **4**. When $FeCl_2$ is used, the route to **11** via **22b** becomes disfavoured. Contrary the yield of **11** reached 25% using the system hemin/L-cysteine.

Rearrangement of **4a** by a 1,3-H-shift (c) gives **16** and in turn **17**. We assume, that the epoxide here is generated in a following step (**18**) leading to **10**. For the formation of **12** (d) we propose the loss of the isopropyl group (with the assistance of O-1) and the addition of an OH radical from the solvent. **13** Is most likely formed by O-2 assisted cleavage of the bond between C-9 and C-10 (e), followed by addition of H radicals from the solvent. However, the conformation of the terpene-A ring in **13** is completely different from the other substances. Irradiation of H-2 gave NOEs to both 1′-H, the methyls at C-3 and at C-1 and an intensity enhancement of the resonance of 2′-H.

4b Rearranges to **8** via two intramolecular radical combinations (similar to **4a**) or via a B-ring opened product to **13** (not shown). Similar reactions and analogous products are observed with peroxide **5**.

Conclusion

We have shown that peroxides **4** and **5** also follow the principles proposed for the Fe^{2+}-induced isomerisation of artemisinin and other naturally occurring peroxides. We therefore conclude that these compounds might also have considerable antimalarial activity.

Experimental

Melting points: melting point apparatus Dr Tottoli, uncorrected. Optical rotation: polarimeter 241 MC (Perkin–Elmer). MS: Varian MAT 711 spectrometer, 70 eV electron impact. IR spectra: infrared spectrometer system 2000 FTIR (Perkin–Elmer). UV–Vis: UV-160A UV–visible recording spectrophotometer (Shimdazu). NMR spectra: Varian Unity Inova 400 (297K), Varian Unity Inova 600 (299K) (**9**), 5 mm tubes, solvent resonance as internal standard. ^1H and ^{13}C resonances were assigned using ^1H, ^1H and ^1H, ^{13}C correlation spectra. For the sake of clarity the numbering schemes in the NMR spectra of **8**, **10**, **12** and **15** correspond to **6**, the others to IUPAC. Before performing the NOE experiments, dissolved oxygen was carefully removed by bubbling Ar through the solutions. Elementary analyses are performed by the Laboratory for Microanalyses, Institute of Physical Chemistry, University of Vienna, Austria. The results were in satisfactory agreement with the calculated values. Irridation: high pressure mercury lamp Hanau–Hochdrucklampe TQ 150 Hg, using a 1% K_2CrO_4 solution as filter. Materials: column chromatography (CC): silica gel Kieselgel Merck 60 (70–230 mesh), pore diameter 60 Å; thin-layer chromatography (TLC): TLC plates (Merck) Kieselgel 60 F_{254}, 0.2 mm, 200×200 mm; the substances were detected in UV light at 254 nm and by spraying with molybdatophosphoric acid and subsequent heating with a heat gun.

Abietic acid was isolated from Sacotan 90®, a fraction of Tallharz, the by-product of cellulose manufacturing.[27] Methyl abietate **6** was prepared using the method of Abad et al.[39] in a yield of 98%. The ester was used without further purification.

Abietinol (7)

A solution of 2.0 g (6.31 mmol) methyl abietate **6** in 100 ml dry ether was added dropwise to a suspension 0.35 g (9.20 mmol) $LiAlH_4$ in 100 ml ether and refluxed for 40 min. Excess of hydride was destroyed by dropwise addition of water. The reaction mixture was acified with 2 N HCl. The organic layer was separated and washed twice with $NaHCO_3$ solution, dried (Na_2SO_4) and evaporated yielding 1.8 g (98%) of **7** as a colourless resin. Data correspond to those of Ref. 40 ($C_{20}H_{32}O$).

Methyl-[1*R*-(1α,4aβ,4bα,7α,9α,10aα)]-4b,7-epidioxy-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodeca-hydro-9-hydroxy-1,4a-dimethyl-7-(1-methylethyl)-1-phenanthrene-carboxylate (4). A solution of 5.0 g methyl abietate **6** (15.5 mmol) and 28 mg methylene blue in 500 ml of 96% ethanol was irradiated with a high pressure mercury lamp for 48 h with a 1% K_2CrO_4 solution as filter while oxygen was bubbled through the solution. The reaction mixture was evaporated and diluted with ether and ethanol. 20 g (133 mmol) NaI and 1 ml AcOH conc. were added and stirred overnight. The solution was concentrated in vacuo, diluted with ether and washed several times with a 10% $Na_2S_2O_3$ solution, 0.2 N NaOH and water, dried (Na_2SO_4) and evaporated. The resulting oil was purified by column chromatography (cyclohexane/ethyl acetate 1:1) yielding 1.16 g of **4** (20%) as a colourless resin. Anal. Calcd for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85; found: C, 69.0; H, 8.81; NMR data agree with those given in lit.[35]

[1*R*-(1α,4aβ,4bα,7α,10aα)]-4b,7-Epidioxy-1-hydroxymethyl-1,4a-dimethyl-7-(1-methylethyl)-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-9-phenanthrol (5). A solution of 160 mg **4** (0.43 mmol) in 20 ml dry ether was added dropwise to a suspension of 0.04 g (1.05 mmol) $LiAlH_4$ in 20 ml ether. The reaction mixture was stirred at room temperature overnight and hydrolysed with an aqueous NH_4Cl solution. The aqueous layer was extracted twice with ether. Combined organic layers were washed with saturated brine, dried (Na_2SO_4) and evaporated yielding 90 mg (61%) of **5** as white crystals, mp 195°C (lit.[35]: 197°C). NMR and MS data agree with those given in lit.[35] ($C_{20}H_{32}O_4$).

A solution of 1.74 g abietinol **7** (6.03 mmol) and 23 mg methylene blue in 200 ml of 96% ethanol was irradiated with a high pressure mercury lamp for 48 h with a 1% K_2CrO_4 solution as filter while oxygen was bubbled through the solution. For work-up the same procedure was used as described for methyl abietate **6**. After purification by column chromatography CH_2Cl_2/MeOH (10:1) 203 mg (10%) of **5** were obtained as white crystals, mp 195°C.

Isomerisation of 4 with ferric chloride (general procedure)

Freshly purchased $FeCl_2{\cdot}4H_2O$ (1 equiv.) was added to **4** (1 equiv.) in MeCN (10 ml). The mixture was stirred for 30 min–2 h at room temperature under Ar and filtered over Celite and silica. The latter was washed with CH_2Cl_2 and AcOEt. Filtrate and washings were combined and evaporated. The residue was purified by column chromatography (silica, cyclohexane/AcOEt 2:1). E.g. after 1 h 250 mg (0.69 mmol) of **4** with 137 mg (0.69 mmol) of $FeCl_2{\cdot}4H_2O$ yielded 40 mg (16 %) **8**, 58 mg (23 %) **9**, 12 mg (5%) **11**, 10 mg **10** and 10 mg **15** (4% each).

Methyl-1a*R*-[1aβ,1bα,2α,3aα,4α,7aβ,7bα,9aβ]-1b,7b-epoxy-perhydro-2-hydroxy-4,7a-dimethyl-9a-(1-methylethyl)-phenanthrene[1,2-b]oxirane-4-carboxylate (8). Colourless resin, R_f=0.54 (cyclohexane/AcOEt=1:1); UV: –; $[\alpha]_D^{22}$=8.07° (c=0.52, CH_2Cl_2); IR (KBr) $\tilde{\nu}$: 3475, 2936, 2874, 1726, 1464, 1253, 1069, 919 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.90 (d, 3H, 16-H*, J=6.9 Hz), 0.92 (d, 3H, 17-H*, J=6.9 Hz), 0.98 (s, 3H, 19-H), 1.02 (m, 1H, 6-H_{eq}), 1.08 (s, 3H, 20-H), 1.35 (m, 1H, 1-H_{ax}), 1.50 (sept, 1H, 15-H, J=6.9 Hz), 1.50–1.60 (m, 2H, 3-H_{eq}, 3-H_{ax}), 1.60 (m, 2H, 2-H_{eq}, 2-H_{ax}), 1.62 (m, 1H, 1-H_{eq}), 1.70–1.76 (m, 2H, 11-H_{eq}, 11-H_{ax}), 1.50–1.68 (m, 2H, 12-H_{ax}, 12-H_{eq}), 1.80 (td, 1H, 6-H_{ax}, J=6.8 Hz, J=13.2 Hz), 2.10 (d, 1H, 5-H, J=13.2 Hz), 2.55 (d, 1H, 7-OH, J=6.7 Hz), 3.45 (s, 1H, 14-H), 4.31 (m, 1H, 7-H) ppm; ^{13}C NMR (100 MHz,

$CDCl_3$): δ 16.28 (q, C-19), 16.46 (q, C-20), 17.13 (q, C-16*), 17.77 (q, C-17*), 18.44 (t, C-2), 21.32 (t, C-11), 23.57 (t, C-12), 28.55 (t, C-6), 34.35 (d, C-15), 35.04 (t, C-1), 36.46 (t, C-3), 36.73 (s, C-10), 40.37 (d, C-5), 47.17 (s, C-4), 52.14 (q, C-21), 54.29 (d, C-14), 59.57 (s, C-13), 60.99 (s, C-8), 68.51 (d, C-7), 71.11 (s, C-9), 179.5 (s, C-21) ppm; $C_{21}H_{32}O_5$, MS (70 eV, EI): *m/z* (%): 346 (2) [M^+-H_2O], 335 (2), 318 (5), 303 (6), 278 (5), 275 (13), 258 (5), 243 (8), 199 (100), 123 (20), 109 (10).

Methyl-1*R*-[2α,3aα,4α,7aα]-spiro-[3′-oxo-6′-(1-methylethyl)-7′-oxabicyclo[4.1.0]$^{1',6'}$heptan-1,2′-[1H]-2,3,3a,4,5,6,7,7a-octahydro-2-hydroxy-4,7a-dimethyl-4-indene-carboxylate] (9). White crystals, mp 181–182°C; R_f=0.50 (cyclohexane/AcOEt =1:1); UV (CH_2Cl_2): λ (log ε)= 229.6 nm (1.795), 224 nm (1.475); $[\alpha]_D^{22}=-67.6$ (*c*=0.37, CH_2Cl_2); IR (KBr) $\tilde{\nu}$: 3522, 2959, 2929, 2870, 1727, 1701, 1463, 1262, 1103 cm^{-1}; ^1H NMR (600 MHz, C_6D_6): δ 0.62 (dt, 1H, 7-H_{eq}), 0.86 (d, 3H, 9′-H*, *J*=6.9 Hz), 0.93 (d, 3H, 10′-H*, *J*=6.9 Hz), 0.95 (td, 1H, 5-H_{ax}, *J*=13.6 Hz, *J*= 4.0 Hz), 0.96 (s, 3H, 8-H), 0.99 (s, 3H, 10-H), 1.32 (dquint, 1H, 6-H_{eq}, *J*=14.4 Hz, *J*=3.2 Hz), 1.38 (td, 1H, 7-H_{ax}, *J*=13.6 Hz, *J*=4.3 Hz), 1.45 (sept, 1H, 8′-H, *J*=6.9 Hz), 1.70 (qt, 1H, 6-H_{ax}, *J*=13.8 Hz, *J*=3.6 Hz), 1.75 (m, 2H, 5′-H_{ax}, 5′-H_{eq}), 1.90 (m, 2H, 4′-H_{eq}, 3-H), 2.00 (m, 1H, 3-H), 2.00 (dt, 1H, 5-H_{eq}), 2.30 (ddd, 1H, 4′-H_{ax}, *J*= 16.4 Hz, *J*=9.1 Hz, *J*=7.3 Hz), 3.05 (dd, 1H, 3a-H, *J*= 11.8 Hz, *J*=9.3 Hz), 3.51 (s, 1H, 1′-H), 5.36 (dd, 1H, 2-H, *J*=9.2 Hz, *J*=3.2 Hz) ppm; ^{13}C (100 MHz, C_6D_6): δ 17.84 (q, C-16), 17.98 (q, C-9*), 19.53 (t, C-6), 20.92 (q, C-10), 21.26 (t, C-5′), 27.15 (q, C-8), 28.96 (t, C-5), 30.59 (t, C-7), 33.72 (t, C-3), 34.59 (d, C-8′), 35.46 (t, C-4′), 43.19 (s, C-4), 47.56 (s, C-7a), 51.17 (q, OCH_3), 62.50 (s, C-6′), 62.77 (d, C-1′), 67.56 (s, C-1(2′)), 73.49 (d, C-2), 177.73 (s, C-9), 208.32 (s, C-3′) ppm; MS (70 eV, EI): *m/z* (%): 364 (10) [M^+], 346 (74), 331 (40), 314 (17), 286 (24), 268 (29), 251 (100), 243 (40), 207 (29), 153 (30), 123 (20), 107 (33); Anal. Calcd for $C_{21}H_{32}O_5$: C, 69.2; H, 8.85; found: C, 69.0; H, 8.79.

Methyl-[1*R*-(1α,4aβ,4bα,7α,8aα,9α,10aα)]-4b,8a-epoxy-tetradecahydro-1,4a-dimethyl-9-hydroxy-7-(1-methylethyl)-8-oxo-1-phenanthrene-carboxylate (10). Colourless resin; R_f=0.77 (cyclohexane/AcOEt=1:1); UV: –; $[\alpha]_D^{22}=-37.26$ (*c*=0.21, CH_2Cl_2); IR (KBr) $\tilde{\nu}$: 3439, 2955, 1873, 1726, 1462, 1386, 1252, 1071 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.78 (d, 3H, 16-H*), 0.88 (d, 3H, 17-H*, *J*=6.9 Hz), 1.07 (s, 3H, 19-H), 1.12 (s, 3H, 20-H), 1.06 (dt, 1H, 6-H_{eq}), 1,50–1,70 (m, 2H, 2-H_{ax}, 2-H_{eq}), 1.52 (m, 2H, 1-H, 3-H), 1.70 (m, 1H, 1-H), 1.60 (dt, 1H, 6-H_{ax}, *J*=4.1 Hz, *J*=10.9 Hz, *J*=13.9 Hz), 1.82 (m, 1H, 3-H), 1.60 (m, 1H, 11-H_{ax}), 1.80 (m, 1H, 11-H_{eq}), 1.80 (m, 1H, 12-H_{eq}), 1.85 (m, 1H, 13-H), 2.20 (m, 1H, 12-H_{ax}), 2.22 (d, 1H, 5-H, *J*=12 Hz), 2.40 (m, 1H, 15-H), 3.20 (s, br, 1H, OH), 3.65 (s, 3H, 21-H), 4.50 (m, 1H, 7-H) ppm; ^{13}C (100 MHz, $CDCl_3$): δ 15.80 (t, C-2), 16.58 (2C, q, C-19,20), 17.79 (q, C-16*), 18.60 (t, C-11), 19.90 (q, C-17*), 23.75 (t, C-12), 26.75 (t, C-6), 28.08 (d, C-15), 34.82 (t, C-1), 35.66 (t, C-3), 37.60 (s, C-10), 40.19 (d, C-5), 47.61 (s, C-4), 51.97 (q, C-21), 53.08 (d, C-13), 65.34 (d, C-7), 65.56 (s, C-9), 74.02 (s, C-8), 179.5 (s, C-18), 210.50 (s, C-14) ppm; $C_{21}H_{32}O_5$, MS (70 eV, EI): *m/z* (%):364 (16) [M^+], 346 (40), 331 (28), 286 (16), 253 (22), 243 (28), 207 (16), 183 (97), 153 (52), 123 (100), 109 (31).

Methyl-1*S*-[2α, 3aα,4α,7aα]-spiro-[3′-oxo-6′-(1-methylethyl)-7′-oxabicyclo[4.1.0.]$^{1',6'}$heptane-1,2′-[1H]-2,3,3a,4,5,6,7,7a-octahydro-2-hydroxy-4,7a-dimethyl-indene-4-carboxylate] (11). White crystals, mp 151–152°C; R_f=0.64 (cyclohexane/AcOEt=1:1); UV (CH_2Cl_2): λ (log ε)= 230.6 nm (2.60), 223.4 nm (2.22); $[\alpha]_D^{21}=-135.0$, (*c*=0.16 CH_2Cl_2), IR (KBr) $\tilde{\nu}$: 3490, 2942, 2877, 1724, 1688, 1462, 1247, 1204, 1198, 1034 cm^{-1}; ^1H NMR (400 MHz, C_6D_6): δ 0.92 (d, 3H, 9′-H*, *J*=6.9 Hz), 0.94 (m, 1H, 5-H_{ax}), 0.96 (m,1H, 7-H_{ax}), 0.98 (d, 3H, 10′-H, *J*=6.9 Hz), 1.02 (s, 3H, 8-H), 1.13 (s, 3H, 10-H), 1.37 (dquint, 1H, 6-H_{eq}, *J*=13.7 Hz, *J*=3.5 Hz), 1.53 (sept, 1H, 8′-H, *J*=6.9 Hz), 1.60 (dm, 1H, 7-H_{eq}, *J*=13.2 Hz), 1.72 (ddd, 1H, 3-H_{eq}, *J*=14.6 Hz, *J*=11.8 Hz, *J*=10.4 Hz), 1.75 (m, 2H, 5′-H_{ax}, 5′-H_{eq}), 1.90 (qt, 1H, 6-H_{ax}, *J*=13.7 Hz, *J*=3.4 Hz), 2.00 (d, 1H, 2-OH, *J*=10.8 Hz), 2.02 (m, 1H, 4′-H_{eq}), 2.06 (ddd, 1H, 3-H_{ax}, *J*=14.6 Hz, *J*=10.4 Hz, *J*=4.5 Hz), 2.10 (dt, 1H, 5-H_{eq}), 2.50 (td, 1H, 4′-H_{ax}, *J*=7.1 Hz, *J*=13.7 Hz), 2.85 (s, 1H, 1′-H), 3.4 (t, 1H, 3a-H, *J*=10.4 Hz), 4.00 (ddd, 1H, 2-H, *J*=11.8 Hz, *J*=10.8 Hz, *J*=4.5 Hz) ppm; ^{13}C (100 MHz, C_6D_6): δ 17.82 (q, C-9′), 18.23 (q, C-10′), 19.38 (t, C-6), 20.75 (q, C-10), 21.95 (t, C-5′), 26. 84 (q, C-8), 29.33 (t, C-5), 29.85 (t, C-7), 34.12 (d, C-8′), 37.09 (t, C-3), 38.63 (t, C-4′), 42.99 (s, C-4), 46.52 (d, C-3a), 49.76 (s, C-7a), 51.12 (q, OCH_3), 61.74 (s, C-6′), 62.43 (d, C-1′), 65.66 (s, C-1), 77.01 (d, C-2), 177.65 (s, C-9), 212.9 (s, C-3′) ppm; MS (EI, 70 eV): *m/z* (%); 346 (8) [M^+-H_2O], 321 (18), 303 (30), 261 (4), 243 (20), 215 (7), 182 (47), 123 (100); Anal. Calcd for $C_{21}H_{32}O_5$: C, 69.2; H, 8.85; found: C, 69.17; H, 8.85.

Methyl-[1*R*-(1α,4aβ,4bα,7α,8aα,9α,10aα)]-4b,8a-epoxy-tetradecahydro-1,4a-dimethyl-7,8,9-trihydroxy-7-(1-methylethyl)-1-phenanthrene-carboxylate (15). Colourless resin; R_f=0.65 (cyclohexane/AcOEt=1:1); UV: –; IR (KBr) $\tilde{\nu}$: 3481, 2931, 1724, 1459, 1389, 1255, 1072, 729 cm^{-1}; ^1H NMR (400 MHz, C_6D_6): d 0.98 (s, 3H, 18-H), 0.98 (d, 3H, 16-H*, *J*=6.9 Hz), 1.10 (s, 3H, 19-H), 1.12 (dt, 1H, 6-H_{eq}), 1.20 (1H, m, 12-H_{ax}), 1.24 (d, 3H, 17-H*, *J*=6.9 Hz), 1.20–1.40 (m, 2H, 1-H), 1.28–1.50 (m, 2H, 2-H), 1.50–1.60 (m, 2H, 3-H), 1.54 (dt, 1H, 11-H_{eq}), 1.56 (1H, m, 12-H_{eq}), 1.74 (ddd, 1H, 6-H_{ax}, *J*=14.0 Hz, *J*=12.2 Hz, *J*=3.2 Hz), 2.14 (sept, 1H, 15-H, *J*=6.9 Hz), 2.18 (td, 1H, 11-H_{ax}), 2.28 (d, 1H, 5-H, *J*=12.1 Hz), 2.35 (d, 1H, 14-OH, *J*=10.8 Hz), 2.45 (d, 1H, 7-OH, *J*=7.0 Hz), 3.50 (s, 3H, 21-H), 4.35 (ddd, 1H, 7-H), 4.90 (dd, 1H, 14-H, *J*=10.8 Hz, *J*=1.7 Hz) ppm; ^{13}C (100 MHz, C_6D_6): δ 16.39 (q, C-18), 16.84 (q, C-19), 17.24 (q, C-16*), 17.42 (q, C-17*), 18.86 (t, C-2), 22.40 (t, C-11), 24.57 (t, C-12), 28.47 (t, C-6), 33.87 (d, C-15), 35.98 (t, C-1), 36.40 (t, C-3), 37.69 (s, C-10), 40.58 (d, C-5), 47.36 (s, C-4), 51.69 (q, C-21), 67.23 (d, C-7), 67.69 (d, C-14), 67.85 (s, C-8), 76.72 (s, C-9), 78.48 (s, C-13), 178.22 (s, C-20) ppm; $C_{21}H_{34}O_6$, MS (70 eV, EI): *m/z* (%): 382 (8) [M^+], 364 (10), 347 (49), 329 (20), 307 (23), 269 (30), 243 (38), 214(33), 189 (28), 183 (39), 175 (87), 147 (41), 123 (100).

Isomerisation of 4 with hemin (general procedure)

Under Ar **4** (1 equiv.) was dissolved in a 1:1 mixture of MeCN/aqueous phosphate buffer (pH 7.8). L-Cysteine (1 equiv.) was added, followed by hemin (0.1–1 equiv.). The mixture was stirred at room temperature for 4–65 h,

quenched with aqueous saturated EDTA, stirred for a few minutes, diluted with water and extracted several times with ether. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography (silica, cyclohexane/AcOEt 2:1).

A (Hemin molar): 230 mg (0.63 mmol) **4**, 10 ml solvent, 430 mg (0.66 mmol) hemin, 81 mg (0.67 mmol) L-cysteine, reaction time 4 h; yield: 21 mg **8** (9%), 48 mg **9** (21%), 51 mg **11** (22%) and 28 mg **13** (12%).

B (Hemin catalytic): 285 mg (0.78 mmol) **4**, 10 ml solvent, 53 mg (0.08 mmol) hemin, 96 mg (0.80 mmol) L-cysteine, reaction time 21 h; yield: 24 mg **8** (9%), 40 mg **9** (14%), 57 mg **11** (20%) and traces of **10**.

C (Hemin catalytic): 282 mg **4**, 10 ml solvent, 52 mg hemin, 96 mg L-cysteine, reaction time 65 h, same results as described above for 21 h and additional 28 mg (10%) of **13**.

Methyl-1*R*,2*S*,2′*R*,3*R*,4″*R*-2-(2′-hydroxy-2′-(2″-($\Delta^{2'',3''}$-4″-hydroxy-4″-(1-methylethyl)-1″-oxo-cyclohexenyl)-ethyl))-1,3-dimethyl-1-cyclohexylcarboxylate (13). Colourless resin; R_f=0.32 (cyclohexane/AcOEt=1:1); UV (MeOH): λ (log ϵ)=231.8 (3.086) nm; $[\alpha]_D^{23}$=13.2° (c=0.038, MeOH); IR (KBr) $\tilde{\nu}$: 3447, 2956, 2874, 1727, 1672, 1468, 1378, 1250, 1145, 733 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.90 (d, 3H, 9-H), 0.96 (d, 3H, 8″-H*, J=6.9 Hz), 1.02 (d, 3H, 9″-H*, J=6.9 Hz), 1.10 (s, 3H, 7-H), 1.04 (m, 1H, 4-H), 1.18 (m, 1H, 6-H_{ax}), 1.28 (m, 1H, 4-H), 1.38 (m, 1H, 5-H), 1.44 (m, 2H, 2x1′H), 1.50 (m, 1H, 5-H), 1.52 (m, 1H, 3-H), 1.78 (dt, 1H, 6-H_{eq}, J=13.6 Hz, J=4.0 Hz), 1.92 (sept, 1H, 7″-H, J=6.9 Hz), 1.94 (m, 1H, 5″-H), 2.10 (m, 1H, 5″-H), 2.12 (d, 1H, 2-H), 2.40 (dt, 1H, 6″-H_{eq}, J=17.2 Hz, J=5.3 Hz), 2.62 (ddd, 1H, 6″-H_{ax}, J=17.2 Hz, J=10.2 Hz, J=5.2 Hz), 3.07 (s, br, 1H, 2-OH, J=7.9 Hz), 3.64 (s, 3H, OCH_3), 4.15 (m, 1H, 2′-H), 6.63 (s, 1H, 3″-H) ppm; ^{13}C (100 MHz, $CDCl_3$): δ 16.32 (q, C-8″*), 17.51 (q, C-9″*), 19.27 (t, C-9), 22.21 (t, C-5), 24.06 (q, C-7), 28.93 (t, C-4), 29.88 (t, C-5″), 31.19 (t, C-6), 32.40 (t, C-1′), 33.18 (d, C-3), 34.57 (t, C-6″), 37.06 (d, C-7″), 40.98 (d, C-2), 48.23 (s, C-1), 51.86 (q, OCH_3), 71.87 (s, C-7″), 72.76 (d, C-2′), 139.98 (s, C-2″), 148.67 (d, C-3″), 178.96 (s, C-8), 200.76 (s, C-1″) ppm; $C_{21}H_{34}O_5$, MS: (70 eV, EI): m/z (%): 366 (7) [M^+], 348 (8), 330 (6), 316 (6), 288 (11), 245 (16), 183 (100), 165 (21), 123 (20), 109 (19).

Isomerisation of 4 with ferric chloride with addition of L-cysteine

Treatment of 211 mg (0.58 mmol) peroxide **4** with 115 mg $FeCl_2 \cdot 4H_2O$ (0.58 mmol) in MeCN/ buffer (at pH 7.8) with addition of 70 mg (0.58 mmol) of L-cysteine at room temperature during 1.5 h gave 38 mg **8** (18%), 41 mg **9** (19%), and the four minor components **10**, **11, 12** and **13** (all about 12 mg (5%) each) after purification by column chromatography (silica, cyclohexane/AcOEt 2:1). The procedure and work-up were the same as described for isomerisation with hemin.

Methyl-[1*R*-(1α,4aβ,4bβ,9α,10aα)]-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-4b,9-dihydroxy-1,4a-dimethyl-7-oxo-1-phenanthrene-carboxylate (12). Colourless resin; R_f=0.08 (cyclohexane/AcOEt=1:1); UV (MeOH): λ (log ϵ)=233.8 (3.947) nm; $[\alpha]_D^{23}$=−51.1° (c=0.035, MeOH); IR (KBr) $\tilde{\nu}$: 3428, 2928, 2249, 1725, 1660, 1459, 1389, 1259, 1131, 1070, 944, 731, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.22 (s, 3H, 19-H), 1.26 (m, 1H, 6-H_{eq}), 1.32 (s, 3H, 18-H), 1.60 (m, 2H, 2-H), 1.50 (m, 1H, 1-H), 1.56 (m, 1H, 1-H), 1.58 (m, 1H, 3-H), 1.66 (m, 1H, 3-H), 1.88 (dd, 1H, 5-H, J=12.0 Hz, J=7.4 Hz), 2.04 (m, 1H, 11-H_{eq}), 2.16 (td, 1H, 11-H_{ax}, J=12.8 Hz, J=4.6 Hz), 2.32 (ddd, 1H, 6-H_{ax}, J=12.6 Hz, J=10.3 Hz), 2.44 (dt, 1H, 12-H_{eq}, J=17.8 Hz), 2.76 (ddd, 1H, 12-H_{ax}, J=17.6 Hz, J=12.3 Hz, J=5.1 Hz), 2.90 (br, 2H, 2×OH), 3.60 (s, 3H, 21-H), 4.80 (ddd, 1H, 7-H, J=8.4 Hz), 6.10 (s, 1H, 14-H) ppm; ^{13}C (100 MHz, $CDCl_3$): δ 17.70 (t, C-2), 18.02 (q, C-19), 18.32 (q, C-18), 32.78 (t, C-11), 33.29 (t, C-6), 33.88 (t, C-12), 34.09 (t, C-1), 37.28 (t, C-3), 39.68 (d, C-5), 42.46 (s, C-10), 46.39 (s, C-4), 52.25 (q, C-21), 65.41 (d, C-7), 73.66 (s, C-9), 122.44 (d, C-14), 168.62 (s, C-8), 178.27 (s, C-20), 199.96 (s, C-13) ppm; $C_{18}H_{26}O_5$, MS (70 eV, EI): m/z (%): 322 (18) [M^+], 304 (47), 290 (22), 272 (16), 245 (26), 227 (25), (32), 189 (27), 123 (100), 109 (95).

Methyl-1*R*,2*S*,2′*R*,3*R*,4″*R*-3-deutero-2-(2′-hydroxy-2′-(2″-($\Delta^{2'',3''}$-4″-hydroxy-4″-(1-methylethyl)-1″-oxo-cyclohexenyl)-ethyl))-1,3-dimethyl-1-cyclohexylcarboxylate (14). The procedure, work-up and purification were the same as described for isomerisation of **4** with hemin. 248 mg (0.68 mmol) **4** Were treated with 90 mg L-cysteine (0.56 mmol) and 142 mg $FeCl_2 \cdot 4H_2O$ (0.72 mmol) in MeCN/ D_2O (14 ml, 1:1) during 1.5 h and yielded 25 mg of **14** after work-up and purification. Colourless resin; R_f=0.32 (cyclohexane/AcOEt=1:1); ^1H and ^{13}C NMR data correspond to **13** except: δ 0.90 (s, 3H, 9-H) ppm.

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