Antimalarial peroxides: the first intramolecular 1,2,4,5-tetraoxane

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An intramolecular steroidal 1,2,4,5-tetraoxane has been synthesised in six steps starting from methyl 3-oxo-7α,12α-diacetoxy-5β-cholan-24-oate. The synthesised 1,2,4,5-tetraoxane has moderate in vitro antimalarial activity against P. falciparum strains (IC50 (D6) = 0.35 μg/mL; IC50 (W2) = 0.29 μg/mL).

Keywords: tetraoxane, malaria, peroxide, steroid, intramolecular.

INTRODUCTION

Tropical malaria, a major health problem in many southern countries, is caused by multiplication of the protozoan parasite Plasmodium falciparum in erythrocytes. More than 400 million disease cases with over 1.5 million deaths are the annual toll of P. falciparum infections. Roll back malaria programs1 are hampered inter alia by the spreading resistance of the parasite to standard antimalarial drugs, in particular to chloroquine (CQ), which had been the affordable and effective antimalarial mainstay for 50 years. The antimalarial properties of artemisinin2 and of other peroxides such as 1,2,4,5-tetraoxacycloalkanes3 opened new avenues in the fight against malaria.

Our research in this area exploited the steroid carrier of cholestane4 and a cholic acid-derived5 series: bis-steroidal tetraoxanes were explored. Now, the synthesis and preliminary antimalarial activity of the first intramolecular steroidal tetraoxane derived from cholic acid is presented.

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RESULTS AND DISCUSSION

Chemistry

The starting ketone 1 was brominated using polymer-bound PyHBr3 (POLY-PyHBr3) in acetic acid at 80 °C (Scheme 1). As expected, the 4β-bromo derivative 2 was obtained (71 %) which was further treated with hydrazine/AcOK6 in order to obtain directly the Δ3-alkene 4 needed for ring-opening to the dialdehyde 6. Unfortunately, the reaction was sluggish and a significant amount of unreacted 2 remained. Thus, alkene 4 was obtained by an alternative route involving the reduction of bromoketone 2 into the epimeric bromohydrine mixture 3 (94 %; 1:1-ratio) which was further treated with Zn/AcOH (69 %). Oxidation of alkene 4 with OsO4/NMO afforded the cis-diol 5 in 90 % yield. Oxidation with LTA/K2CO3 afforded the desired dialdehyde 6 in 87 % yield, which was immediately used in the next step to avoid its relatively fast polycondensation even at low temperatures.

The peroxyacetalization reaction was carried out in a toluene / ethanol / water mixture using 32 % H2O2 as described earlier. Based on spectral data, it was concluded that the obtained tetraoxane 7 is a monomeric compound, i.e., the intramolecular tetraoxane was obtained. Thus, the 13C-NMR spectrum excluded the existence of aldehyde functionality (confirmed in 1H-NMR spectrum) and the two distinct C signals at 108.9 and 106.6 ppm are indicative for peroxyacetal carbons (with differing neighbours). This, together with MS (TOF) and m.a. data is sufficient for assigning the structure of 7 (Fig. 1).

Antimalarial activity

Tetraoxane was screened against Plasmodium falciparum D6 and W2 clones. D6 is a clone from the Sierra I/UNC isolates and is susceptible to chloroquine and pyrimethamine,
but has reduced susceptibilities to mefloquine and halofantrine. W2 is a clone of the Indochina I isolate and is resistant to chloroquine and pyrimethamine, but susceptible to mefloquine.

The activity of 7 against both P. falciparum clones (IC$_{50}$ (D6) = 0.35 µg/mL; IC$_{50}$ (W2) = 0.29 µg/mL) is rather low as compared to other steroidal tetraoxanes of cholic acid and artemisinin (IC$_{50}$ (D6) = 2.37 ng/mL; IC$_{50}$ (W2) = 2.06 ng/mL) but, interestingly, it is more active against CQ resistant W2 clone, exhibiting similar IC$_{50}$ (W2)/IC$_{50}$ (D6) ratio (RI = 0.83) as artemisinin (RI = 0.82).

In conclusion, for the first time, an intramolecular steroidal tetraoxane with medium antimalarial activity has been developed which gives a whole range of possibilities for further structure (and activity) development, particularly of incorporation of other functionalities into the steroid core and by altering the position of the tetraoxane moiety.

**EXPERIMENTAL**

**General**

Melting points were determined on a Boetius PMHK apparatus and were not corrected. Specific rotations were determined on a Perkin-Elmer 141-MC instrument at the given temperatures. IR spectra were recorded on a Perkin-Elmer spectrophotometer FT-IR 1725X. $^1$H- and $^{13}$C-NMR spectra were recorded on a Varian Gemini-200 spectrometer (at 200 and 50 MHz, respectively) in the indicated solvent using TMS as the internal standard. Chemical shifts are expressed in ppm (δ) values and coupling constants (J) in Hz.

EI and CI mass spectra were recorded on a MS Finnigan-MAT 8230 spectrometer with double focusing reverse geometry, using isobutane (CI). Electrospray ionisation mass spectra were acquired on a QStar Pulsar (Applied Biosystems) quadrupole-orthogonal time of flight (QqQ-TOF) hybrid instrument. The samples were dissolved in pure acetonitrile (HPLC grade) to obtain a solution at 1.5 pmol/µL concentration, which was directly introduced into the ion source using a built-in syringe pump. Spectrum acquisition was made in the positive ion mode in the mass range of m/z 300–1500 using the following ion source parameters: 5500 V accelerating voltage, 100 V orifice voltage and 40 V skimmer voltage. The resolution of the instrument in the TOF-MS mode was measured to be 8500 at 50 % valley definition.
Thin-layer chromatography (TLC) was performed on precoated Merck silica gel 60 F254 plates, using N,N-dimethyl-\(p\)-phenylenediammonium dichloride reagent for peroxide moiety detection.\(^7\) Lobar Lichroprep Si 60 (40–63 \(\mu\)m) columns coupled to a Waters RI 401 detector were used for column chromatography.

**Methyl 4\-bromo-3-oxo-7\(\alpha\)-diacetoxy-5\(\beta\)-cholan-24-oate (2)**

A mixture of 1 (10.0 g, 20 mmol) and polymer bound PYbBr\(\text{2}\) (10.0 g, 20 mmol; Fluka) in 500 mL glacial acetic acid was stirred for 1 hour at 80 °C. Then the reaction mixture was cooled, diluted with water, left overnight, and filtered. The remaining solid was dissolved in CHCl\(\text{3}\), and after removal of the polymer the crude product was purified by flash chromatography (eluent: toluene/\(\text{EtOAc}\) = 85/15). Yield 8.30 g (71 %), m.p. = 221–223 °C (colourless prisms, benzene-hexane).

**Methyl 4\-bromo-3-hydroxy-7\(\alpha\)-diacetoxy-5\(\beta\)-cholan-24-oate (3)**

Soluble Na\(\text{BH}_4\) (0.60 g, 16 mmol) was added over 30 min to an ice-cooled mixture of 2 (6.40 g, 11 mmol) in MeOH (120 mL) under stirring. The reaction was quenched by 50 % acetic acid and poured onto an ice / water mixture. After filtration and drying, the crude product was obtained (6.10 g, 94 %) and used in the next step without further purification. 3-Hydroxy epimers (3a and 3b) were separated only for analytical purposes by column chromatography (Lobar B, Lichroprep Si 60, eluent: heptane/\(\text{EtOAc}\) = 8/2).

**Methyl 4\-bromo-3-hydroxy-7\(\alpha\)-diacetoxy-5\(\beta\)-cholan-24-oate (3a)** M.p. = 169–172 °C (colourless prisms, ether-hexane). IR (KBr): 3507, 3493, 3483, 3495, 3235, 2954, 2876, 1734, 1735, 1420, 1445, 1370, 1248, 1027 cm\(^{-1}\). \(\text{\(\delta\)}\)NMR (200 MHz, CDCl\(\text{3}\)): 5.32 (d, \(J = 12.0\) Hz, H-C(4)), 5.13 (s, H-C(12)), 5.07 (d, \(J = 2.8\) Hz, H-C(7)), 3.67 (s, CH\(_2\text{O}_2\text{C}(24)\)), 2.13 (s, CH\(_3\text{O}_2\text{C}(24)\)), 2.17 (s, CH\(_3\text{O}_2\text{C}(24)\)), 2.12 (s, CH\(_3\text{O}_2\text{C}(24)\)), 1.08 (s, H-C(13)), 0.82 (d, \(J = 6.2\) Hz, H-C(2)(10)), 0.78 (s, H-C(13)). \(\text{\(\delta\)}\)C-NMR (50 MHz, CDCl\(\text{3}\)): 174.5, 170.4, 75.3, 70.5, 66.8, 51.5, 47.3, 45.0, 43.8, 43.4, 38.6, 37.9, 34.6, 37.9, 34.5, 30.8, 30.7, 29.4, 28.7, 28.2, 27.1, 26.6, 25.7, 23.2, 22.6, 21.7, 21.3, 17.4, 12.2.

**Methyl 4\-bromo-3-hydroxy-7\(\alpha\)-diacetoxy-5\(\beta\)-cholan-3-ene-24-oate (4)**

To a solution of 3 (6.50 g, 11 mmol) in glacial AcOH (190 mL) and H\(\text{2O}\) (9 mL), Zn powder (37.5 g) was added within 30 min at 45–50 °C. The reaction mixture was vigorously stirred until completion of the reaction. After cooling and filtration, the solvent was removed under reduced pressure. The remaining viscous oil was dissolved in CHCl\(\text{3}\), worked-up in the usual manner, dried over anh. Na\(\text{SO}_4\) and evaporated to dryness. The crude product was purified by flash chromatography (eluent: toluene/\(\text{EtOAc}\) = 9/1) and crystallised from hexane to afford alkene 4. Yield 3.70 g (69 %), m.p. = 116–118 °C (colourless prisms, hexane).

\(\text{[\text{\(\alpha\)l]}_{\text{D}}\text{]}\) = 18.78 (c = 1.00, CHCl\(\text{3}\)). IR (KBr): 3512, 3499, 3483, 3495, 3235, 2954, 2876, 1734, 1420, 1445, 1370, 1248, 1027 cm\(^{-1}\). \(\text{\(\delta\)}\)H-NMR (200 MHz, CDCl\(\text{3}\)): 5.09 (d, \(J = 9.8\) Hz, H-C(3)), 3.66 (s, CH\(_2\text{O}_2\text{C}(13)\)), 2.17 (s, CH\(_3\text{O}_2\text{C}(24)\)), 2.12 (s, CH\(_3\text{O}_2\text{C}(24)\)), 1.08 (s, H-C(13)), 0.82 (d, \(J = 6.2\) Hz, H-C(2)(10)), 0.78 (s, H-C(13)). \(\text{\(\delta\)}\)C-NMR (50 MHz, CDCl\(\text{3}\)): 174.5, 170.4, 75.3, 70.5, 66.8, 51.5, 47.3, 45.0, 43.8, 43.4, 38.6, 37.9, 34.5, 30.8, 30.7, 29.4, 28.7, 28.2, 27.1, 26.6, 25.7, 23.2, 22.6, 21.7, 21.3, 17.4, 12.2.

**Methyl 4\-bromo-3-hydroxy-7\(\alpha\)-cholan-24-oate (5)**

A mixture of 1 (10.0 g, 20 mmol) in glacial AcOH (190 mL) and H\(\text{2O}\) (9 mL), Zn powder (37.5 g) was added within 30 min at 45–50 °C. The reaction mixture was vigorously stirred until completion of the reaction. After cooling and filtration, the solvent was removed under reduced pressure. The remaining viscous oil was dissolved in CHCl\(\text{3}\), worked-up in the usual manner, dried over anh. Na\(\text{SO}_4\) and evaporated to dryness. The crude product was purified by flash chromatography (eluent: toluene/\(\text{EtOAc}\) = 85/15). Yield 8.30 g (71 %), m.p. = 116–118 °C (colourless prisms, hexane), lit. m.p. 115–116 °C.\(^7\) Lobar Lichroprep Si 60 (40–63 \(\mu\)m) columns coupled to a Waters RI 401 detector were used for column chromatography.
Methyl 3β,4β-dihydroxy-7α,12α-diacetoxy-5β-cholan-24-oate (5)

To a solution of alkenne 4 (100 mg, 0.2 mmol) in an acetone / water mixture (4 mL; 4 / 1, v / v) were added NMOH·H2O (110 mg, 0.8 mmol) and a 2.5 % solution of OsO4 in t-BuOH (0.1 mL, 0.01 mmol). The mixture was stirred at room temperature until completion of the reaction, followed by addition of NaHSO3 (10 mg) and further stirring for a further 30 min. The acetone was removed under reduced pressure, brine was added and the water layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na2SO4, and evaporated to dryness. The crude product was purified by column chromatography (Lobar, Lichroprep Si 60, eluent: heptane / EtOAc = 7/3) to afford the dihydroxy compound 5. Yield 96 mg (90 %), m.p. = 83–85 °C (colourless prisms, hexane-EtOAc), lit m.p. 74–76 °C.7

13C-NMR (50 MHz, CDCl3): 174.6, 170.6, 170.5, 75.4, 71.1, 133.0, 123.4, 75.6, 70.7, 51.5, 47.3, 44.9, 43.1, 37.9, 37.1, 35.4, 34.5, 32.1, 31.8, 30.8, 30.6, 27.0, 26.3, 25.6, 22.8, 21.4, 21.2, 20.0, 17.4, 14.0, 12.1. ESI-MS (m/z, %): 625.24 (M+K+)H2O2, 609.28 (M+Na+H2O2), 603 (M+NH4+H2O2), 575.27 (M+Na+, 8), 467.24 (6), 413.25 (3), 284.32 (100).

Methyl 7α,12α-diacetoxy-3,4-dioxo-3,4-seco-5β-cholan-24-oate (6)

To a stirred suspension of 5 (500 mg, 0.96 mmol) and anhydrous K2CO3 (280 mg, 2.0 mmol) in dry benzene (20 mL) under argon, Pb(OAc)4 (440 mg, 0.99 mmol) was added in small portions within 1 h. The mixture was stirred at room temperature until completion of the reaction, followed by addition of NaHSO3 (10 mg) and further stirring for a further 30 min. The benzene was removed under reduced pressure, brine was added and the water layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na2SO4, and evaporated to dryness. The crude product was purified by column chromatography (Lobar, Lichroprep Si 60, eluent: heptane / EtOAc = 3/7) and crystallized to afford the dihydroxy compound 6. Yield 96 mg (90 %), m.p. = 83–85 °C (colourless prisms, hexane-EtOAc), lit m.p. 74–76 °C.7

13C-NMR (50 MHz, CDCl3): 174.7, 171.0, 170.6, 108.9, 106.6, 75.1, 69.8, 51.5, 44.8, 43.3, 43.1, 37.9, 37.1, 35.4, 34.5, 32.1, 31.8, 30.8, 30.6, 27.0, 26.3, 25.6, 22.6, 21.4, 21.2, 20.0, 17.4, 14.0, 12.1. ESI-MS (m/z, %): 625.24 (M+K+)H2O2, 609.28 (M+Na+H2O2), 603 (M+NH4+H2O2), 575.27 (M+Na+, 8), 467.24 (6), 413.25 (3), 284.32 (100).

Methyl 7α,12α-diacetoxy-3,4-dioxo-3,4-seco-5β-cholan-24-oate (7)

To an ice-cooled mixture of 30 % H2O2 (0.23 mL, 2.1 mmol), EtOH (1.40 mL), H2O (1.32 mL) and conc. H2SO4 (2.54 mL), a pre-cooled solution (ice-bath) of dialdehyde (20 mL) under argon, Pb(AcO)4 (440 mg, 0.99 mmol) was added in small portions within 1 h. The mixture was stirred at room temperature until completion of the reaction, followed by addition of NaHSO3 (10 mg) and further stirring for a further 30 min. The acetone was removed under reduced pressure, brine was added and the water layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na2SO4. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (Lobar, Lichroprep Si 60, eluent: heptane / EtOAc = 3/7) to afford the dihydroxy compound 6. Yield 453 mg (87 %). IR (film): 3020m, 2956s, 2877m, 1733s, 1441m, 1378m cm–1. 1H-NMR (200 MHz, CDCl3): 5.08 (s, H-C(12)), 0.74 (s, CH3-C(20)), 0.76 (s, CH3-C(10)).13C-NMR (50 MHz, CDCl3): 174.6, 170.6, 170.5, 75.1, 69.8, 51.5, 44.8, 43.3, 43.1, 37.9, 37.1, 35.4, 34.5, 32.1, 31.8, 30.8, 30.6, 27.0, 26.3, 25.6, 22.6, 21.4, 21.2, 20.0, 17.4, 14.0, 12.1. Anal. calcd. for C29H44O6 (488.67): C 71.28, H 9.08. Found: C 70.64, H 8.60 %.
Animalarial activity

The in vitro antimalarial drug susceptibility screen is a modification of the procedures first published by Desjardins et al.,10 with modifications developed by Milhous et al.11 In brief, the assay relies on the incorporation of radiolabeled hypoxanthine into the parasites. The inhibition of isotope incorporation is attributed to the activity of known or candidate antimalarial drugs. For each assay, proven antimalarials are used as controls. The incubation period is 66 hours and the starting parasitemia is 0.2 % with 1 % hematocrit. The media used is RPMI-1640 culture media with no folate or p-aminobenzoic acid (PABA) and 10 % normal heat inactivated human plasma. For quantitative in vitro drug susceptibility testing, two well-characterized P. falciparum malaria clones are normally used, W2 and D6.12 W2 is a clone of the Indochina I isolate and is resistant to chloroquine and pyrimethamine, but susceptible to mefloquine. D6 is a clone from Sierra I/UNC isolates and is susceptible to chloroquine and pyrimethamine, but has reduced susceptibilities to mefloquine and halofantrine.

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REFERENCES

8. The procedure described here (4 → 5 → 6) is more elaborate than direct ozonolysis of 4 into 6. The ozonolysis afforded only 5–10 % yield of dialdehyde 6, probably due to polycondensation reactions