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A Short Synthesis of Antimalarial Peroxides

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Abstract—A concise and efficient synthesis of two simplified diastereomeric analogues of a natural peroxide is presented. Both compounds could be isolated in high purity and fully identified. They exhibited moderate antimalarial activity.

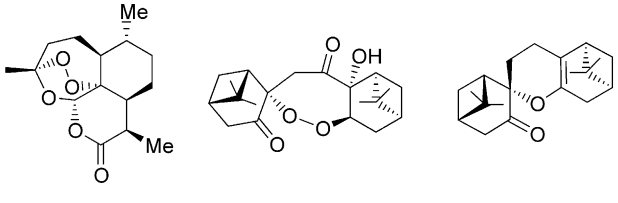
Introduction

Malaria is the most threatening tropical disease causing two million deaths per year and extends its endemic zone in particular to the south of USA and Europe. Nearly all of the reported lethal cases are caused by *Plasmodium falciparum*. Considering the widespread emergence of strains resistant to usual antimalarial drugs, there is a crucial need for an alternative therapy.[1] Endoperoxides are promising antimalarial agents for multidrug-resistant strains. The prototype Artemisinin (**1**, Fig. 1), a natural sesquiterpene with a 1,2,4-trioxane moiety, was isolated from *Artemisia annua*.[2] This, together with some hemisynthetic derivatives, are used in several countries as an alternative therapy for multidrug-resistant cases.

In 1995 a new terpenic peroxide of structure **2** (Fig. 1) was isolated from Cardamom, a fruit from *Amomum krervanh Pierre*.[3] It exhibited a potent in vitro antimalarial activity against *P. falciparum* ($IC_{50}=170$ nM). Its relative configuration was determined by X-ray diffraction analysis, while the absolute configuration was infered from the stereochemistry of known co-isolated terpenes. So far, no synthesis nor partial approach has been reported. To our knowledge, its main functionality pattern, for example, 4-hydroxy-5-oxo-1,2-dioxepane bearing a keto function on C-7 carbon is original and unique. In addition bipinane terpenes are rare; aritazone[4] (**3**, Fig. 1) and its enantiomer Cedronellone[5] are the only known natural products containing this skeleton. This challenge together with our program on antimalarial peroxides[6] prompted us to envisage both the synthesis of natural product **1**, and of simpler derivatives.

Results and Discussion

We report herein an original and short preparation of antimalarial peroxides **9a** and **9b**, two analogues of natural cardamom peroxide **2**, Scheme 1. Thus, organocopper derivative of bromoethylmethyldioxolane **4**[7] was prepared by an halogen-metal exchange with *t*-BuLi[8] followed by transmetallation with CuI. The use of Riecke magnesium[9] as in the Helquist's procedure[10] resulted in predominant formation of the Wurtz dimer. Addition to pinocarvone **5**[11] performed with TMSCl[12] afforded 1,4-adduct **6** as the sole product. Curiously,



1 2 3

Figure 1.

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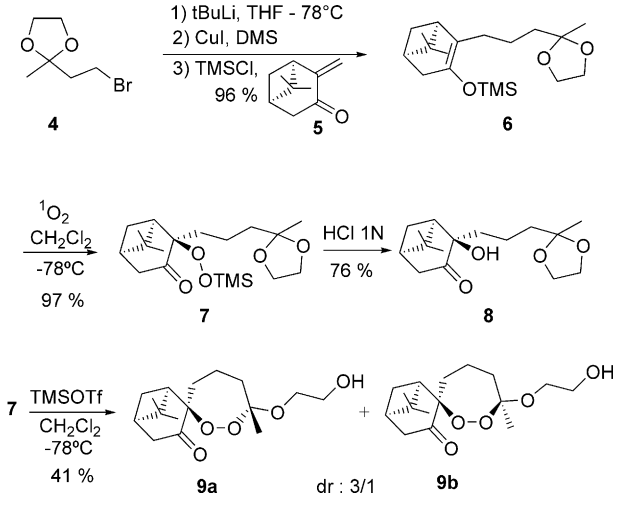
this trimethylsilylenolether **6** showed high stability toward hydrolysis and could be purified by chromatography on silicagel without notable decomposition. Silylenolether **6** was photooxygenated to give, as previously reported for other silylenolethers,[13] the α keto silylperoxide **7** in quantitative yield and with excellent diastereoselectivity.[14] It was anticipated that singlet oxygen approached on the less hindered face,[13b,c] therefore in an *anti* manner to the pinane gem-dimethyl function. Confirmation of the stereochemistry of **7** was obtained later on the synthesis. Since the α-keto silylperoxide **7** decomposed into alcohol **8** under either acidic or protic conditions (1N HCl, MeOH or silicagel), it was straight engaged in the next step without purification. Presumably, desilylation occurred, leading to an unstable α-keto hydroperoxide.[15] Cyclization of the silylperoxide function onto dioxolane was performed successfully using catalytic amount (20% molar) of TMSOTf at −78 °C, affording perketal **9a** and **9b** in a 3:1 diastereomeric ratio. Previous synthesis of artemisinin by cyclization onto acetal of α-silylperoxy aldehyde, an unisolated intermediate resulting from ozonolysis of vinylsilane, has already been reported.[16] But in these conditions (H_2SO_4 3M, SiO_2, CH_2Cl_2), α-silylperoxy ketone **7** completely failed to cyclize. Moreover, trying to perform this reaction with a few other Lewis acids ($BF_3.OEt_2$, $TiCl_4$, $Ti(OiPr)_4$) didn't give the expected product **9a** and **9b**. To the best of our knowledge, this kind of TMSOTf catalyzed cyclization onto ketal has never been realized with α-silylperoxy ketones.

Diastereomers **9a** and **9b** were separated by chromatography on silicagel and fully NMR analyzed (COSY, HMQC, HMBC). In both structure, H_{12a} showed a net deshielding on ^1H NMR that have been attributed to the anisotropic effect of the carbonyl function. Molecular modeling showed later that H_{12a} was in the anisotropic cone of these structures (Figs 2 and 3). This identification of H_{12a} and H_{12b} have then allowed the differentiation of geminal protons H_{10a-b} and H_{11a-b}. NOESY experiments, in $CDCl_3$ and C_6D_6 for each diastereomer, gave us stereochemical confirmation of their structures that was corroborated with molecular modeling (Fig. 2). For both diastereomers **9a** and **9b**, a significant nOe between $H_{12a,b}$ and H_{15} confirmed their previously anticipated 6*R* configuration, so that the peroxide function was *anti* to the gem-dimethyl group. Thus, **9a** and **9b** were epimer at C_9. For both diastereomer, absolute configuration at C_9 has been also determined by NOESY experiment and confirmed with molecular modeling. For compound **9a**, a strong nOe appeared in both solvents between H_{11b} and Me_{16} or H_5 and a weak nOe between Me_{16} and H_5 that is consistent with a 9*R* configuration (Fig. 2). For **9b**, in both solvents, a strong nOe was also observed between H_{11b} and H_5, but Me_{16} showed a net effect with H_{12a} that confirmed its 9*S* absolute configuration.

Two thousands conformations of each compounds, that is, the **9a** and **9b** were generated by random search Monte Carlo method[17] and optimized by TNCG Truncated Newton molecular mechanics minimization method[18] using the Macromodel (version 5.5) program[19] with the MM2 force field.[20] The search was carried out on blocks of 200 Monte Carlo steps until no additional conformation was found to be of lower energy than the current minimum. From these conformational searches, all the possible conformations within 3 kcal/mol from the global minimum were considered. Out of the most stable conformations, for each diastereomer **9a** and **9b**, two were retained for their consistence with nOe and ^1H NMR data: conformers 5E (272.43 kJ/mol) and 7E (273.34 kJ/mol) for **9a**, conformers 1E (265.85 kJ/mol) and 3E (269.96 kJ/mol) for **9b** (Fig. 2). Each differ from their O_7 O_8 puckering that induce too subtle modification on the rest of the molecule to discriminate them.



Scheme 1.

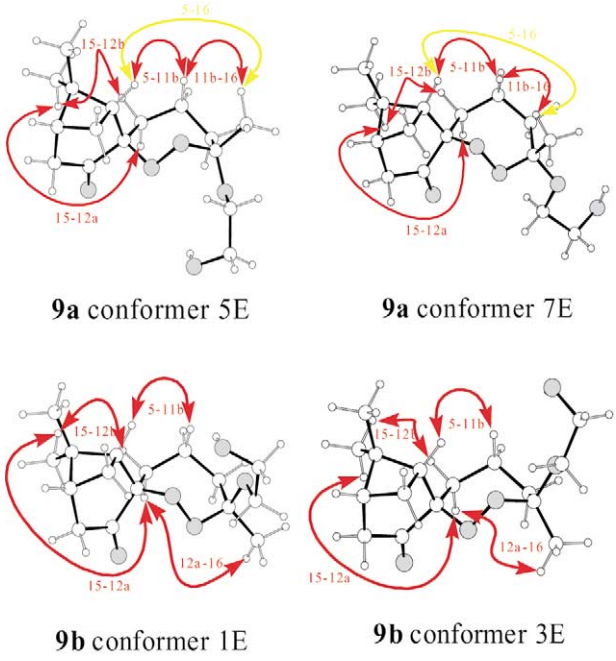


Figure 2. Characteristic NOESY on diastereomer 9a and 9b for their two energy minimized conformer. Strong nOe are represented with red arows and weak nOe with yellow ones. Numbers indicate between wich protons were observed the nOe.

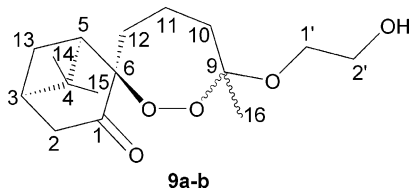


Figure 3. Numbering compounds **9**. Protons with number followed with 'a' are down and with 'b' are up in this representation.

Antimalarial activities was evaluated in vitro against FCR3 *P. falciparum*,[21] a strain wich shows low sensitivity to artemisinin (IC_{50} = 55 nM). Diastereomer **9a** was the most active one with an IC_{50} of 1.05 μM that was one nineteenth of artemisinin potency. The other diastereomer **9b** revealed less active with an IC_{50} = 2.7 μM.

Conclusion

This is the first reported synthesis of an analogue cardamom peroxide **2**. The antimalarial activities of the more potent synthetic diastereomer and of the natural product are quiet similar, compared to artemisinin (respectively one nineteenth and one tenth[3]). This original and short synthetic scheme could be extended to the synthesis of closer analogues of the natural product.

Experimental

NMR experiments were conducted on a Brüker AC 200 and a Brüker ARX 400 spectrometer. Elemental analyses were performed on a Perkin–Elmer 2400 analyser. Silicagel was supplied from Merck (Geduran 230–400 mesh). (1*S*)-α-pinene was purchased from Aldrich $[\alpha]_D = -50.7°$ (neat). THF and CH_2Cl_2 were dried over sodium/benzophenon and CaH_2, respectively. Commercial anhydrous DMS was used (Aldrich). Desoxygenated solvants were obtained bubbling through argon during 15 mn prior to use. Ammonia buffer refer to a 9:1 mixture of respectively saturated NH_4Cl solution with 30% ammonia solution.

(1*R*)-2-[3-(2-methyl-[1,3]dioxolan-2-yl-propyl]-3-trimethylsilyloxy-6,6-dimethyl-bicyclo [3.1.1]hept-2-ene (6). To a dry and argon-sparged solution of bromoethylmethyldioxolane **4**[7] (600 μL, 4.35 mmol) in THF (10 mL) at −78 °C was added over 30 min 1.5 M *tert*-BuLi (4.3 mL, 6.1 mmol). After 5 min more, an argon degased anhydrous solution of CuI (415 mg, 2.1 mmol) in a 1:5 mixture of dimethylsulfide and THF was slowly injected. The yellow reaction mixture turned dark brown. This was treated after 20 min at −78 °C with TMSCl (275 μL, 2.17 mmol) and then (1*R*)-pinocarvone **5**[11] (170 μL, 1.08 mmol). The reaction was quenched after 30 min by adding ammonia buffer (20 mL) and Et_2O (20 mL), and stirred one h at room temperature. Decanted organic phase was washed with ammonia buffer until the aqueous phase turned blue. Combined aqueous phases were extracted with Et_2O (10 mL), organic phases were washed with brine (10 mL), dried over $MgSO_4$ and solvents were evaporated. The oily residue was purified by flash chromatography on silicagel (petroleum ether/CH_2Cl_2: 2/3) to give 350 mg (96%) of silylenolether **6** as a colourless oil. $[\alpha]_D = 0.0$ (c = 1.81, $CHCl_3$); IR (film, cm^{-1}): 1672, 1196, 937, 840; ^1H NMR (200 MHz, $CDCl_3$): δ 3.96–3.78 (m, 4H), 2.30 (dt, *J* = 8.5, 5.5 Hz, 1H), 2.23–2.17 (m, 2H), 2.07–1.85 (m, 4H), 1.54–1.47 (m, 2H), 1.41–1.25 (m, 2H), 1.24 (s, 3H), 1.20 (s, 3H), 1.11 (d, *J* = 8.5 Hz, 1H), 0.82 (s, 3H), 0.14 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 140.8, 124.0, 110.2, 64.5, 43.7, 40.8, 39.6, 39.2, 35.7, 33.1, 29.6, 26.3, 23.7, 21.9, 21.2, 0.9. Anal. calcd for $C_{19}H_{34}O_3Si$: C, 67.41; H, 10.12. Found: C, 67.18; H, 10.01.

(1*R*,2*R*)-2-[3-(2-methyl-[1,3]dioxolan-2-yl-propyl]-3-trimethylsilylperoxy-6,6-dimethyl-bicyclo[3.1.1]heptan-3-one (7). A solution of 900 mg of enolether **6** (2.66 mmol) and 5 mg of methylene blue in CH_2Cl_2 (100 mL) at −78 °C was oxygenated by bubbling through oxygen and irradiated with 400W sodium lamp for 30 min. Then, the solution was warmed up to room temperature, dried over $MgSO_4$, and the solvent was evaporated to dryness under reduced pressure to give 985 mg of crude silylperoxide **7** as a violet oil. This couldn't be furthermore purified without decomposition and so, was analysed and used as a crude mixture. ^1H NMR (200 MHz, $CDCl_3$): δ 3.9–3.6 (s, 4H), 2.55–2.41 (m, 2H), 2.40–1.82 (m, 4H), 1.75–1.45 (m, 10H), 0.8 (s, 3H), 0.10 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 206.4, 110.0, 88.3, 64.4, 45.1, 43.6, 40.3, 39.3, 38.4, 30.9, 27.3, 27.2, 23.7, 22.5, 17.0,−1.3.

(1*R*,2*R*)-2-hydroxy-2-[3-(2-methyl-[1,3]dioxolan-2-yl-propyl]-6,6-dimethyl-bicyclo[3.1.1] heptan-3-one (8). 1 mL of 1N HCl was added to a solution of 385 mg of crude silylperoxide **7** (1.04 mmol) in THF (15 mL) at 0° C. After 2 h at room temperature, satd $NaHCO_3$ solution (2 mL) was added. The reaction mixture was extracted with CH_2Cl_2 (2×10 mL). The combined organic layers was dried over $MgSO_4$ and the solvents evaporated under reduced pressure. Purification by flash chromatography on silicagel (CH_2Cl_2/MeOH:8/2) gave 223 mg (76% Yield) of alcohol **8** as an oil. IR (film, cm^{-1}): 3460, 1718; ^1H NMR (200 MHz, $CDCl_3$): δ 4.0–3.9 (m, 4H), 2.65–2.55 (m, 2H), 2.5–2.4 (m,1H), 2.2 (t, *J* = 5.9 Hz, 1H), 2.1–2.15 (bs, 1H), 2.05 (ddd, *J* = 8.9 Hz, *J* = 5.9 Hz, *J* = 2.5 Hz, 1H), 1.85 (td, *J* = 12.4 Hz, *J* = 3.1 Hz, 1H), 1.7–1.55 (m, 4H), 1.37 (s, 3H), 1.31 (s, 3H), 0.92 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 213.6, 110.2, 78.2, 64.6, 45.9, 43.2, 39.4, 38.4, 38.2, 36.3, 28.4, 27.5, 23.9, 22.8, 17.1. Anal. calcd for $C_{16}H_{26}O_4$: C, 68.06; H, 9.28. Found: C, 68.31; H, 9.17.

(5*R*,6*R*,9*R*)-9-(2-Hydroxyethoxy)-4,4,9-trimethyl-3,5-methano-7,8-dioxaspiro[5.6] dodecan-1-one (9a) and (5*R*,6*R*,9*S*)-9-(2-Hydroxyethoxy)-4,4,9-trimethyl-3,5-methano-7,8-dioxaspiro[5.6]dodecan-1-one (9b). A solution of 985 mg of crude silylperoxide **7** (2.66 mmol) in anhydrous CH_2Cl_2 (100 mL) at −78° C under argon, was treated with TMSOTf (100 μL, 0.55 mmol). After 2 h, the reaction was quenched with Et_3N (200

μL, 1.43 mmol), washed twice with saturated $NaHCO_3$ solution (25 mL), dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. The crude oil was purified by chromatography on silicagel (CH_2Cl_2/Et_2O: 3/1) to give 245 mg of endoperoxide **9a** (Fig. 3) (31%) and 79 mg of its diastereomer **9b** (10%). **9a**: $[\alpha]_D = -4.3$ (c = 1.62, EtOH); IR (film, cm^{-1}): 3479, 1702, 1460, 1371, 868; ^1H NMR (400 MHz, $CDCl_3$): δ 3.95–3.78 (m, 1H, $H_{2'}$), 3.72–3.63 (m, 2H, $H_{1'}$ $H_{2'}$), 3.62–3.50 (m, 1H, $H_{1'}$), 2.66–2.57 (m, 3H, H_{2a} H_{2b} H_5), 2.50 (ddt, $J_{13a-13b} = 10.7$ Hz, $J_{13a-5} = J_{13a-3} = 6.2$ Hz, 1H, H_{13a}), 2.37 (ddd, $J_{12b-12a} = 15.8$ Hz, $J_{12b-11b} = 11.9$ Hz, $J_{12b-11a} = 2.8$ Hz, 1H, H_{12b}), 2.34 (br s, OH), 2.18 (t, $J_{10a-10b} = J_{10a-11b} = 12.4$ Hz, $J_{10a-11a} = 2.8$ Hz, 1H, H_{10a}), 2.15–2.08 (m, 1H, H_3), 1.92–1.75 (m, 2H, H_{11a}, H_{10b}), 1.84 (d, $J_{13b-13a} = 10.7$ Hz, 1H, H_{13b}), 1.66–1.54 (m, 1H, H_{11b}), 1.51 (ddd, $J_{12a-12b} = 15.8$ Hz, $J_{12a-11b} = 8.5$ Hz, $J_{12a-11a} = 3.5$ Hz, 1H, H_{12a}), 1.39 (s, 3H, H_{14}), 1.22 (s, 3H, H_{16}), 0.82 (s, 3H, H_{15}); ^{13}C NMR (100 MHz, $CDCl_3$): δ 205.4 (C_1), 108.0 (C_9), 91.3 (C_6), 63.0 ($C_{2'}$), 62.2 ($C_{1'}$), 45.7 (C_5), 43.3 (C_2), 40.0 (C_{10}), 38.6 (C_4), 38.1 (C_3), 34.9 (C_{12}), 27.8 (C_{13}), 27.5 (C_{14}), 22.8 (C_{15}), 20.2 (C_{16}), 19.2 (C_{11}); Mass spectrum (MALDI), *m*/*z*: 321.163 (MNa^+). Anal. calcd for $C_{16}H_{26}O_5$: C, 64.41; H, 8.78. Found: C, 64.77; H, 8.92.

9b: (Fig. 3) $[\alpha]_D = +142.9$ (c = 0.175, EtOH); IR (film, cm^{-1}): 3527, 1718, 1456, 1372, 868; ^1H NMR (400 MHz, C_6D_6): δ 3.8–3.7 (m, 2H, $H_{1'}$ $H_{2'}$), 3.65–3.55 (m, 1H, $H_{2'}$), 3.32–3.21 (m, 1H, $H_{1'}$), 2.51 (ddd, $J_{12b-12a} = 14.3$ Hz, $J_{12b-11a} = 9.7$ Hz, $J_{12b-11b} = 1.2$ Hz, H_{12b}), 2.45 (ddd, $J_{2a-2b} = 18.5$ Hz, $J_{2a-3} = 3.1$ Hz, $J_{2a-13a} = 2.8$ Hz, 1H, H_{2a}), 2.38 (dd, $J_{2b-2a} = 18.5$ Hz, $J_{2b-3} = 2.0$ Hz, 1H, H_{2b}), 2.10 (dtd, $J_{13a-13b} = 11.0$ Hz, $J_{13a-5} = J_{13a-3} = 7.1$ Hz, $J_{13a-2a} = 2.8$ Hz, 1H, H_{13a}), 2.05–1.95 (m, 1H, $H_{10b,}$), 1.92 (dd, $J_{5-6a} = 8.4$ Hz, $J_{5-3} = 6.4$ Hz, 1H, H_5), 1.86 (d, $J_{13b-13a} = 11.0$ Hz, 1H, H_{13b}), 1.78–1.58 (m, 3H, H_{11a},H_{11b}, H_{10a}), 1.53 (tt, $J_{3-5} = J_{3-13a} = 6.2$ Hz, $J_{3-2a} = J_{3-2b} = 3.1$ Hz, 1H, H_3), 1.27 (ddd, $J_{12b-12a} = 14.3$ Hz, $J_{12b-11b} = 8.1$ Hz, $J_{12b-11a} = 2.1$ Hz, 1H, H_{12b}), 1.05 (s, 3H, H_{16}), 0.91 (s, 3H, H_{14}), 0.52 (s, 3H, H_{15}); ^{13}C NMR (100 MHz, $CDCl_3$): δ 205,4 (C_1), 107.8 (C_9), 88.5 (C_6), 63.0 ($C_{2'}$), 62.2 ($C_{1'}$), 47.0 (C_5), 43.6 (C_2), 40.7 (C_{10}), 38.9 (C_3), 36.4 (C_4), 32.5 (C_{12}), 27.4 (C_{13}), 27.1 (C_{14}), 22.7 (C_{15}), 20.9 (C_{16}), 18.4 (C_{11}); Mass spectrum (MALDI), *m*/*z*: 321.161 (MNa^+). Anal. calcd for $C_{16}H_{26}O_5$: C, 64.41; H, 8.78. Found: C, 64.65; H, 8.82.

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