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Alkylation of natural endoperoxide G3-factor. Synthesis and antimalarial activity studies†

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Alkylation of the peroxyhemiketal function is described and all synthesised endoperoxides show good antimalarial activity. New rearrangement reactions in the presence of $CsCO_3$, and preliminary results on Fe(II) chemical reduction of the O–O bond are presented.

New, cheap antimalarial agents, which can act for example in a way similar to artemisinin[1–5] or other endoperoxides, are still the subject of intensive research. We focused on the synthesis of modified endoperoxide G-factors, in order to understand and possibly to improve their antiplasmodial activities. G3-factor is a natural endoperoxide involved in plant defence[6] easily extracted from the leaves of *Eucalyptus grandis*, a species widely used for paper pulp. We previously reported the crucial role of the peroxyketal function for antimalarial activity in the G-factor series.[7] We found that G3Me (Fig. 1), the methyl ether analogue of G3, was about one hundred-fold more active than G3 on *Plasmodium falciparum*, while after acetylation it had only a weak activity, in the same range of values as G3 (IC_{50} = 20.5 μM on Nigerian strain). When OH or OMe was replaced by a fluoride, despite the presence of the peroxo bridge, the compound was no longer active (IC_{50} >100 μM).[7]

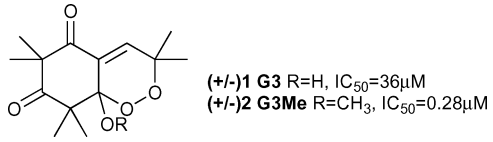
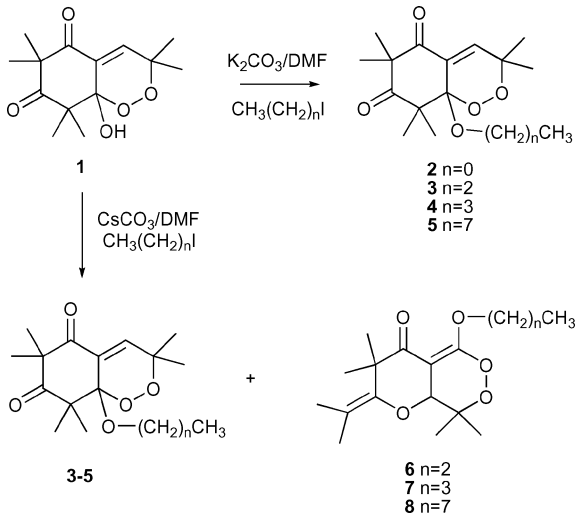


Fig. 1 G3-factor and its methyl ether.

We wish to describe in this report our results on the synthesis and antiplasmodium activities of different alkoxylated analogues. Preliminary results concerning the biomimetic Fe(II) induced reduction of one analogue are also reported.

Although alkylation of a tertiary hydroxyl group is considered to be a classical reaction, it appeared to be rather difficult in this series. As previously described,[8] methylation did not occur in acidic media. A methyl moiety was introduced in drastic conditions: butyllithium in THF then methyl triflate. Methyl iodide or dimethyl sulfate were inefficient in these conditions. This methodology cannot be extended to other groups. Use of sodium or potassium hydrides or potassium hexamethyldisilazide led to degradation products even in the presence of powerful electrophiles like mesylate or monochlate (monochloromethanesulfonate). Finally, use of potassium carbonate allowed G3-alkylation on the hemiketal function. With alkyl iodides, potassium carbonate in excess in suspension in DMF, allows the reaction to occur, even slowly, and after 48 h leads to the alkyl endoperoxides **2–5** in 70–30% yield from **1**, after purification (Scheme 1). It is likely that alkylation is observed with potassium because the bond length is greater than with the other cations used. It should allow a solvent separated ion pair and a more dissociated alkoxide to be formed which will react with alkyl iodides.



Scheme 1 Introduction of an aliphatic chain.

This method provides a first access to the expected compounds and an alternative and easier way to endoperoxide G3Me **2**.

When Cs_2CO_3 was used instead of K_2CO_3, the yields (about 20% for **3–5**) were not improved and new products **6–8** (about 8%) were formed. As they were systematically obtained, whatever the chain length of the aliphatic alkyl iodides used, they were isolated and identified by NMR analysis[9] (^1H, ^{13}C, HMBC, HSQC correlation) and mass spectroscopy. Typical NMR data, and especially heteronuclear multiple bond connectivity, are given in the electronic supplementary information† and were used for attribution of an enol ether structure to compound **7**, (**6, 8**). Based on this connectivity, O-alkylation takes place on the ketonic carbonyl α to the *gem*-dimethyl group (in preference to the ester-like carbonyl α to the peroxide bridge).

The unexpected rearrangement observed can tentatively be explained as follows: a retro-aldol reaction affording the enolate (b) then O-cyclisation in a Michael type addition, assisted by the Cs^+ cation, takes place to give (c), that is then alkylated yielding the final rearranged product (Scheme 2). It is likely that the rearrangements are only observed with caesium because this cation can form a chelated complex with the two carbonyl functions and consequently activates the unsaturated system

† Electronic supplementary information (ESI) available: experimental procedure, ^1H and ^{13}C NMR data, mass spectroscopy, IR spectroscopy for compounds **3–5, 7, 9, 10**, ^1H and ^{13}C spectra, HMBC, HSQC, COSY, correlations for compound **7** and chiral separation chromatogram for compounds **9a, 9b**. See http://www.rsc.org/suppdata/ob/b5/b503402g/

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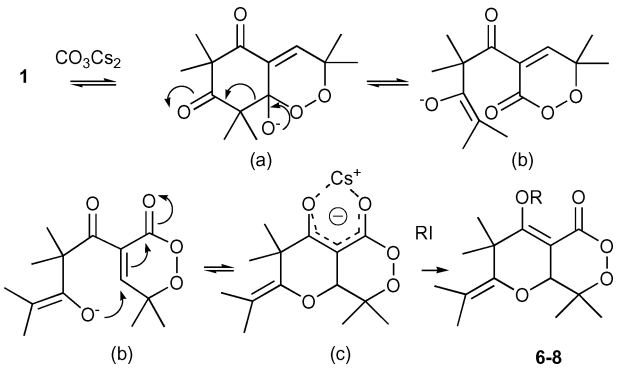
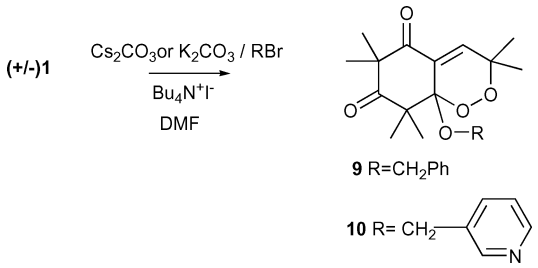


Scheme 2 Conceivable mechanism for the formation of compounds **6–8**.

leading to Michael-type cyclisation. The amount of direct O-alkylation and "rearranged" compounds depends on the relative rates of alkylation.

Benzylation was accomplished using either K_2CO_3 (5 eq) or Cs_2CO_3 (2 eq) in DMF, and benzyl bromide with catalytic amounts of Bu_4NI. After 48 h, benzyl endoperoxide **9** was obtained in respectively 33% and 42% yield after purification (Scheme 3). Similarly, compound **10** was obtained when using methyl-pyridine bromide hydrobromide as a reactant (10% yield). In both cases, no rearranged products, similar to **6–8**, were observed in the presence of $CsCO_3$.



Scheme 3 Introduction of the benzyl and methyl pyridine groups.

The desired endoperoxides **3–5, 9, 10**, and one of the rearranged compounds **7**, were then tested *in vitro* against the Nigerian strain of *Plasmodium falciparum*.[10] The activity was determined according to the method of Desjardins[11] by the use of [^{3}H] hypoxanthine incorporation as an assessment of parasite growth.

Parasitic viability was expressed as IC_{50} which is the drug concentration causing 50% parasite growth inhibition. Results are consigned in Table 1.

First of all, it can be noted that all of the compounds alkylated on the peroxyketal function are active against *Plasmodium*, in the same range of values (<1 μM), which is good, though not yet comparable to artemisinin. The chain lengthening does not significantly improve the biological activity, indicating that only alkylation is the main factor of their improved activity in comparing with non-alkylated G3 endoperoxide.

The influence of the chirality of the hemiketalic center on the activities was also examined in one example. Separation of the two enantiomers of benzylated endoperoxide **9** was successful using the chiral column CHIRACEL OD. The $[\alpha]_D$ of each enantiomer was measured: **9a** $[\alpha]_D^{25} = 3.36$ (*c* 0.5 in $CHCl_3$), **9b** $[\alpha]_D^{25} = -3.47$ (*c* 0.5 in $CHCl_3$). Both enantiomers keep the same IC_{50}: thus the chirality of the hemiketalic center appears to have no influence on the antiplasmodium activity. It is not surprising since Yingzhaosu and Arteflene, which are good antimalarial compounds, present a reverse absolute configuration of the carbon α to the O–Of bond.[12,13] Besides, the activity of BO7 was not influenced by the absolute configuration of the molecule, the pure enantiomers being no more active than the racemate.[14]

Despite the absence of the ketal function in its structure, it can be noted that the rearranged compound **7** possessed a weak activity, similar to that of G3 but far less than that of the alkylated compounds **2–5** or of the benzylated one **9**. We then performed tests for some of our compounds on other *Plasmodium falciparum* strains, which are chloroquine sensitive 3D7-, highly chloroquine resistant FCM29-Cameroon and chloroquine and pyrimethamine resistant W2-Indochina. The results are summarized in Table 2. Compounds **2–10** were active on both chloroquine resistant and sensitive strains with, however, better values on the resistant strains, which is also known for artemisinin-like compounds such as the trioxaquines of Meunier.[15] Benzylated endoperoxide **9** presented an IC_{50} of 100 nM on highly resistant chloroquine strains which indicates a really interesting activity.

In order to understand how endoperoxides act against plasmodium, benzylated compound **9** was used to examine the redox properties. The electrochemical behaviour of compound **9** was studied using a thin layer voltammetry method under potentiostatic conditions as described previously.[16] The E_p value for benzyl endoperoxide is −1.78 V, a value similar to that of G3Me (−1.76 V) and artemisinin (−1.68 V). Integration of the peak showed a one-electron exchange according to the mechanism already described for this family of endoperoxides.

A chemical approach to the reduction process was then undertaken, using Fe(II) complexes as reducing agents.

As done earlier for G3 and G3Me,[17] we studied the biomimetic Fe(II) induced reduction of compound **9** and characterized their degradation products. The conditions chosen were $FeSO_4/CH_3CN–H_2O$, as for G3 or G3Me, because they are closer to biological conditions. After consumption of endoperoxide **9**, three major compounds were isolated: **11, 12** and **13**, in a 1 : 1 : 1 proportion. A proposed mechanism is presented in Scheme 4. After one-electron transfer from Fe(II) to the endoperoxide bridge and homolytic breaking of the O–O bond, the O-centered radical rearranges to give a C-centered radical: a more stable tertiary *gem*-dimethyl carbon radical. This radical can react in different ways: it can either

Table 2 *In vitro* antimalarial activity on different strains

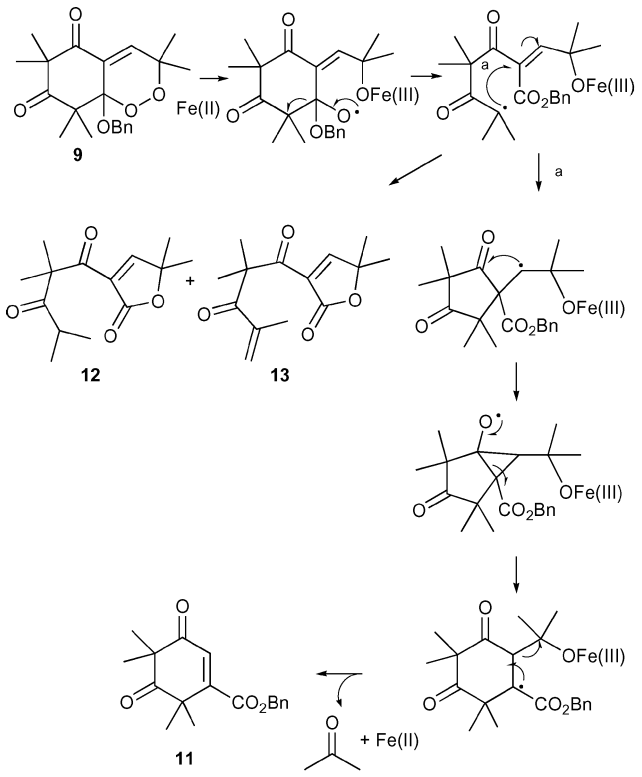
	$IC_{50}/\mu M^a$ for the various strains			
Compound	Nigerian[10]	3D7	FCM29	W2
1	36	62	41	38.50
2	0.28	3.30	0.23	0.23
4	0.76	1.04	0.19	0.44
9	**0.21**	**0.37**	**0.10**	**0.20**
10	0.28	0.82	0.12	0.33
Artemisinin	0.008	0.019	0.004	0.004
Chloroquin	0.03	0.019	0.155	0.125

[a] IC_{50} values are duplicated and were considered acceptable when values did not vary by more than a factor of three.

Table 1 *In vitro* antimalarial activity on Nigerian strains

	1	**2**	**3**	**4**	**5**	**7**	**9**	**9a**	**9b**	**10**
$IC_{50}/\mu M^a$	36	0.28	0.16	0.76	0.18	26	0.21	0.36	0.34	0.28

[a] IC_{50} values are duplicated and were considered acceptable when they did not vary by more than a factor of three.



Scheme 4 Conceivable mechanism for the reduction of **9** by Fe(II).

become engaged in a 5-*exo*-trig intramolecular cyclisation giving benzylic ester **11** after acetone elimination and Fe(II) expulsion, or it can disproportionate, yielding lactones **12** and **13**. This tertiary radical could present alkylating properties since besides intramolecular rearrangement, it also gives rise to intermolecular reactions during disproportionation. An alternative way could be the generation of reactive oxygen species (ROS) within the parasitized erythrocyte.[18] This could explain its good biological properties. Further insight into this mechanism and its implications in antiplasmodial activity will be reported elsewhere.[17]

Acknowledgements

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9 **7** ^{1}H NMR (400 MHz) δ_{ppm}: 0.97 (t, CH_3), 1.39 (s, 6H, $2CH_3$), 1.41 (m, 2H, CH_2), 1.51 (s, 6H, $2CH_3$), 1.57 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.67 (m, 2H, CH_2), 4.14 (t, 2H, CH_2), 6.42 (s, 1H, CH); ^{13}C NMR (100.6 MHz) δ_{ppm}: 13.98 (CH_3), 19.10 (CH_3), 19.38 (CH_3), 19.40 (CH_2), 25.29 ($2CH_3$), 27.09 ($2CH_3$), 30.85 (CH_2), 47.03 (C_q), 65.30 (CH_2), 82.48 (C_q), 121.12 (C_q), 126.80 (CH), 144.18 (C_q), 148.15 (C_q), 167.21 (C_q), 176.91 (C_q). MS (DCI/NH_3): m/z (%) = 325 $[MH]^+$ (29.7), 342 $[MNH_4]^+$ (100).
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17 **11** ^{1}H NMR (250 MHz) δ_{ppm}: 1.35 (s, 6H, $2CH_3$), 1.53 (s, 6H, $2CH_3$), 5.07, 5.12 (*AB system*, 2H, CH_2), 6.83 (1H, s, CH), 7.38, 7.21 (5H, *m*, CH arom.). **12,13** were also observed after G3Me reduction. They are fully described in another paper (submitted for publication).
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