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Crucial role of the peroxyketal function for antimalarial activity in the G-factor series

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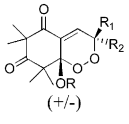
Abstract—New endoperoxides, related to the natural phytohormones known as G factors (G1, G2, G3), were modified on the side chain and the ketalic position. An unexpected rearrangement, specific to one diastereoisomer was observed in the deprotection step of *O*-silylated compounds and attributed to a hexacoordinated fluorosilicon intermediate. The reduction potential of these new peroxides was determined. They exhibited good to moderate antimalarial activity, greatly related to the presence of peroxyketal function.

1. Introduction

G factors are natural endoperoxides originally extracted from the leaves of *Eucalyptus grandis*. They are known to be involved in plant defence (frost resistance, drought stress) and can also act as growth regulators and phytohormones.[1] We have previously reported an optimised synthesis of the G factors and some analogues[2] which were found to possess enhanced antimalarial activities. The parent compounds were obtained in a two-step procedure i.e., Mannich reaction between syncarpic acid and the corresponding aldehyde, then acid hydrolysis of the Mannich base followed by spontaneous time-modulated oxygen uptake leading to the expected endoperoxides. Methylation of the tertiary hydroxyl group under basic conditions can lead to the fully protected endoperoxides (Fig. 1). Better antimalarial activity was obtained for these protected compounds.[3]

Malaria is the most widespread infection disease. It kills one million children and afflicts 300 million people every year. New drugs are urgently needed because conventional cheap treatments have become compromised by drug resistance.[4]

In connection with our work on the structure–activity relationships of this class of antimalarial compound, we evaluated the influence of a hydroxyl group positioned in the R^1 (or R^2) side-chain of endoperoxides **1**–**4** and that of new modifications on the hemiketalic position. Literature reported examples of potent antimalarial acyclic peroxides[5] and bicyclic sulfonyl endoperoxides.[6]



	R	R^1	R^2
G1, G2	H	Me/Et	Et/Me
G3	H	Me	Me
G1Me, G2Me	Me	Me/Et	Et/Me
G3Me	Me	Me	Me
1	Me	Me	$CH_2OSitBu(Ph)_2$
2	Me	$CH_2OSitBu(Ph)_2$	Me
3	H	Me	$CH_2OSitBu(Ph)_2$
4	H	$CH_2OSitBu(Ph)_2$	Me

Figure 1.

Keywords: Malaria; Endoperoxides; G-factor; Hexacoordinated silicon compounds.
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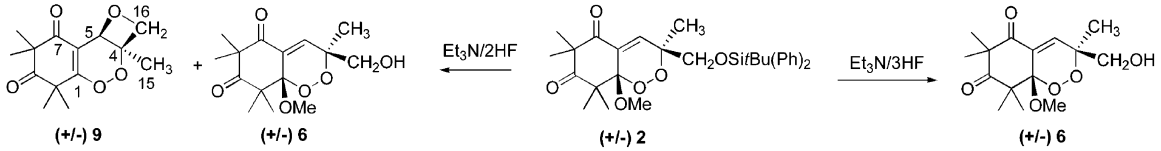
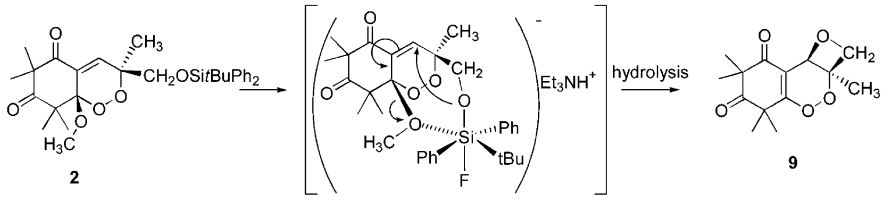


Scheme 1.



Scheme 2.

We wish to highlight in this report of our findings on this subject and the role of the peroxyketal function in the antimalarial activity.

2. Synthesis

Our first aim was related to the role of a supplementary hydroxyl function which could be obtained from racemic compounds **1**–**4**. Although rather classical and expected to be easy, removal of the *tert*-butyl diphenyl silyl group proved to be difficult.

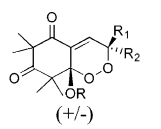
Deprotection of compound **1** was first performed in the presence of tetrabutyl ammonium fluoride adsorbed on silica gel. The reaction was carried at room temperature and followed by thin-layer chromatography. Even after a prolonged period (1 week), deprotection was achieved in low yield and conversion never exceeded 50%. Similar results were obtained when using a THF solution of tetrabutyl ammonium fluoride (TBAF 1M/THF).

We then tried triethylamine/hydrogen fluoride complexes, also known to remove silyl groups. The reaction was found to be dependent both on the stoichiometry between HF and Et_3N and on the nature of the diastereoisomer to be deprotected. For compound **1** the $Et_3N{\cdot}3HF$ complex gave identical results to TBAF. Only 50% conversion was obtained after one week of reaction. In contrast, when using $Et_3N{\cdot}2HF$ complex (15 equivalents, 48 h), deprotected compound **5** was obtained in 80% yield after silica gel purification. The $Et_3N{\cdot}2HF$ complex produces fluoride anions which are claimed to be more nucleophilic than in $Et_3N{\cdot}3HF$.[7] It was obtained, according to a known procedure,[7] by addition of triethylamine to $Et_3N{\cdot}3HF$ ($2Et_3N{\cdot}3HF + Et_3N \rightarrow 3\ Et_3N{\cdot}2HF$).

Surprisingly, diastereoisomer **2** behaved in a completely different way. Removal of the silyl group was found to be optimal when using a solution of $Et_3N{\cdot}3HF$ complex (10 equiv, 1 week), affording compound **6** in 43% yield after silica gel purification. On the other hand, when using a solution of $Et_3N{\cdot}2HF$ complex, starting compound **2** more rapidly gave a mixture of compounds. After silica gel purification, deprotected compound **6** was obtained in low yield ($<10\%$) along with tars and a major adduct which was identified spectroscopically to be the rearranged compound **9** (30% yield) (Scheme 1).[8]

In order to account for this novel rearrangement, a transient hexacoordinated fluorosilicon species[9] can tentatively be proposed (Scheme 2). This species might be formed in the presence of the better 'F^-' nucleophile that is $Et_3N{\cdot}2HF$. In the case of diastereoisomer **2** the oxygen atom of the methoxyl group can stabilize the electron-deficient silicon atom that acquires significant Lewis acid character after fluorine incorporation. Literature data[9] and molecular modelling[10] strongly suggest an apical position for the fluorine atom in the hexacoordinated silicon species. Cleavage of the apical Si–O bond initiates an addition–elimination leading to tricyclic compound **9**. The proposed mechanism could explain why only one diastereoisomer undergoes this peculiar rearrangement. It is worth mentioning that the tricyclic compound **9**, while lacking the peroxy ketal function, possesses an enone frame adjacent to a endoperoxidic linkage and a constrained four-membered cyclic ether.

O-Silylated endoperoxides **3** and **4**, possessing a tertiary hydroxyl function were then deprotected. Compound **3** was deprotected successfully using the $Et_3N{\cdot}2HF$ complex. The reaction was carried at room temperature for 30 h, yielding compounds **7** and **8** in 56% yield after silica gel purification (Fig. 2). The two diastereoisomers were obtained during the purification step as an inseparable mixture (30/70). Compound **4** was deprotected more easily by using 10 equivalents of $Et_3N{\cdot}3HF$ (7 h, rt) in 53% yield. Isomerisation also occurred during purification on silica gel affording the two diastereoisomers **7**, **8** in 53% overall yield (30/70).



	R	R^1	R^2
5	Me	Me	CH_2OH
6	Me	CH_2OH	Me
7	H	Me	CH_2OH
8	H	CH_2OH	Me

Figure 2.

Finally, in order to evaluate the influence of the hydroxyl (or methoxyl) group of the bridgehead frame on antiplasmodial activity, we prepared compounds possessing a fluorinated or acetylated function. Fluoride **10** was prepared classically by reaction of compound G3 with diethylaminosulfurtrifluoride (DAST) and was obtained in 50% yield. Acetoxy compound **11** was prepared by reaction of endoperoxide G3 with acetic anhydride/Et_3N in CH_2Cl_2 (Scheme 3).

3. Electrochemical studies and comparison with antimalarial activities

The electrochemical behaviour of endoperoxides **5–11** was studied and compared with artemisinin, using a thin layer voltammetry method[11] under potentiostatic conditions as described previously.[3] E_p values are reported in Table 1. The results indicate behaviour similar to that of the parent compounds previously reported. In particular, integration of the peak showed a one-electron exchange, suggesting that a radical anion appears at the electrode[12] according to the equation:

$$AB + e^- \rightarrow (AB)^{\circ -} \rightarrow A^{\circ} + B^-$$

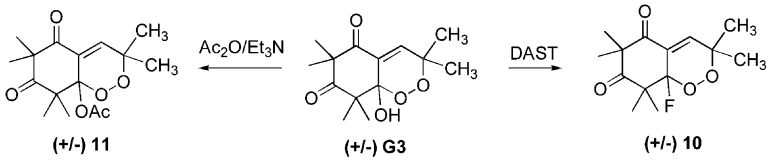
The E_p reduction potentials of the O–O bond vary with the nature of the functions substituting the tertiary hydroxyl group of the hemiketal system. Introduction of the fluorine atom strongly increases the reduction potential of the O–O bond. The E_p value of **10** is found to be −1.32 V in comparison with G3 (−1.50 V). The acetylated compound does not seem to be affected. In contrast, the E_p potential was greatly enhanced for the rearranged compound **9** where the C=C bond is adjacent to the peroxidic linkage. Finally compounds **5** and **6** present values approaching that of G3. Interestingly, these values are differently modified in comparison with the silylated endoperoxides (**3** and **4** respectively)[3]. Compound **6** possesses a higher anodic value in comparing to diastereoisomer **5**. Molecular modelling shows[10] that only for compound **6** ($d_{O\text{-}H..Oend} = 2.11$ Å), can a hydrogen bond occur between the hydroxyl group and one oxygen atom of the endoperoxidic ring. Consequently, the potential peak of O–O bond reduction shifts towards anodic values.

Compounds **5–11** were tested in vitro against the Nigerian strain of *Plasmodium falciparum*. The activity was determined according the method of Desjardins et al.[13] by 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. The results can be found in Table 1.

Compounds **7** and **8** that have been dihydroxylated are no longer active (IC_{50} >100μM) perhaps due to their additional hydroxyl group (compared to G3). Neither fluoride **10** or tricyclic compound **9** are active (IC_{50} >100μM); their E_p values are not in the optimum range (near that of artemisinin or G3Me); fluoride **1** is easily reduced, while tricyclic adduct **9** is too cathodic. Dihydroxylated compounds **7** and **8** are no longer active (>100μM). The acetylated compound **11** presents an activity similar to natural G3. Finally a great difference in the antimalarial activity was observed between diastereoisomers **5** and **6** while this was not the case for fully protected endoperoxides **1** and **2**. It should be pointed out that this was consistant with the striking modification of their E_p values.

4. Conclusion

New endoperoxides belonging to the G-factor family have been synthesized. Modifications have been introduced on the hemiacetal position and on the side chain by removal of the silyl groups, which appeared to be dependent on the source of the fluoride ion, and on the diastereoisomer used. An unusual rearrangement was observed when desilylating diastereoisomer **2** leading to an original tricyclic compound. The reduction potentials of the O–O bonds were determined and the antimalarial activities of all compounds were evaluated. The results indicated that the peroxyketal function strongly influences antiplasmodial activity and that redox properties are another parameter to be considered. Work is in progress to introduce new substituents onto the



Scheme 3.

Table 1. Reduction potential E_p of endoperoxides and in vitro antimalarial activity on Nigerian strains

	Artemisinin	G3	G3Me	1	2	5	6	7/8	10	11	9
IC_{50} μM[a]	0.015	36	0.28	1.5	1.4	1.4	40	>100	>100	20.5	>100
E_p/V[b]	−1.68	−1.50	−1.76	−1.70	n.d.	−1.50	−1.41	−1.56	−1.32	−1.56	−2.13

[a] IC_{50} values were considered acceptable when values did not vary by more than a factor of three.
[b] vs SCE (±0.01 V).

hemiketalic position. Considering the natural G1–G3 compounds these modifications may provide easy access to semisynthetic compounds of low cost.

Acknowledgements

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8. ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 5.26 (s, 1H, C*H* in position 5), 5.25 (d, 1H, $^{2}J_{Hb\text{-}Ha} = 0.86$ Hz, CH_2 in position 16), 4.76 (d, 1H, $^{2}J_{Ha\text{-}Hb} = 0.86$ Hz, CH_2 in position 16), 1.78 (s, 3H, CH_3 in position 15), 1.48 (s, 3H, CH_3 in position 14), 1.41 (s, 3H, CH_3 in position 13), 1.364 (s, 3H, CH_3 in position 12), 1.361 (s, 3H, CH_3 in position 11). ^{13}C NMR (100.64 MHz, $CDCl_3$): δ [ppm] = 212.47 (C_9), 194.38 (C_1), 181.18 (C_7), 117.56 (C_4), 107.58 (C_6), 94.05 (C_{16}), 82.32 (C_5), 56.10 (C_8), 45.66 (C_{10}), 24.84 (C_{12}), 24.66 (C_{11}), 24.34 (C_{14}), 24.08 (C_{13}), 21.35 (C_{15}). IR (neat): ν [cm^{-1}] = 1657(C=O), 809 (O–O). MS (DCI/NH_3): *m*/*z* (%) = 267 $[MH]^+$ (100), 284 $[MNH_4]^+$ (71).
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10. Molecular mechanics calculations were run with Insight II (release 98;0) program using Discover 3 calculation mode (MSI, San Diego, USA). Consistence valence force fields (CVFF) were used. Energetically optimised conformations were determined by sampling structures during molecular dynamic simulations. They were run for 10 ps at 500K using the NVT method, with a 1 fs time step. From the energy/time curves, six to seven minima were chosen and minimised. The same iterative operations (dynamic simulations and minimisation) were undertaken on these minima until no energy variation occurred. For each minimisation rms < 0.001.
11. All the electrode potentials were measured with respect to a saturated calomel electrode (SCE i.e., $Hg/Hg_2Cl_2/Cl^-$ saturated), immersed in a Luggin capillary located near (3–4 mm) the working electrode and containing DMF and the electrolyte in large excess. The auxiliary electrode was made of platinum. The electrochemical apparatus used was a Radiometer Voltalab PGZ 100 computerised potentiostat. The electrochemical cell was kept in a chamber under an inert atmosphere (nitrogen at 1.5 atm) and all experiments were performed in the absence of oxygen.
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