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New Readily Accessible Peroxides with High Anti-Malarial Potency

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Abstract—In an explorative study for new anti-malarial substances using the methyl esters (**1** and **2**) of peroxyplakoric acids A_3 and B_3 as scaffolds, 6-carbomethoxymethyl-3-methoxy-3-pentyl-1,2-dioxane, which has been readily synthesized from 6-keto-α,β-unsaturated ester, was found to exhibit potent anti-malarial activity with high selective toxicity.

In the last two decades, malaria has regained its status as a serious threat to the health and economic prosperity of the human race. It is estimated that approximately 300 million clinical cases were observed and people in excess of 2.5 million die from this disease each year. Due to resistance of the vector (*Anopheles* mosquito) to insecticides and an ongoing spread of the drug-resistant strains of *Plasmodium falciparum* against chloroquine and other clinically used drugs, exploration of new effective anti-malarials has been needed urgently.[1]

In the previous paper, we reported two potent anti-malarial spongean peroxides, the methyl esters (**1** and **2**) of peroxyplakoric acids A_3 and B_3.[2,3] Furthermore, a facile construction method for 6-carbomethoxymethyl-3-methoxy-1,2-dioxane (**3**), the core structure of **1** and **2**, was developed.[3] In our continued program exploring for more potent anti-malarials by use of **1** and **2** as scaffold substances, we have undertaken an accessible synthesis of two congeners (**5** and **6**). This paper communicates the synthetic method of **5** and **6** and an assessment of their anti-malarial potency.

When 6-carbomethoxymethyl-3-methoxy-3-methyl-1,2-dioxane (**3**) (a mixture of diastereomers; **3a**:**3b** = 4.8:1)[3] was evaluated for inhibitory effect on proliferation of *P. falciparum*, the anti-malarial effect of **3** was significantly reduced as compared with those of **1** and **2** (Table 2). Similarly, another peroxide relative to **1** and **2**, chondrillin (**4**)[4] isolated from a marine sponge of *Xestospongia* sp., was shown to display weaker anti-malarial activity as well as low selective toxicity. These findings allowed us to presume that the anti-malarial potency of **1** and **2** involving high selective toxicity would be related to three functionalities: a 1,2-dioxane ring, a conjugated diene portion in a side chain, and a methyl residue adjacent to a carbomethoxyl group (Fig. 1).

As a preliminary investigation on participation of the three functions, we designed accessible 6-carbomethoxymethyl-3-methoxy-1,2-dioxane analogues possessing linear side chains on C-3. On the basis of cLogP,[5,6] which is an effective parameter to predict both permeability and in vivo biological activity of drugs, two analogues were designed as shown in Figure 2. Namely, one analogue **6** possesses nearly as large cLogP as **1** and **2**, the other **5** has cLogP that is considered to belong to the range for exerting in vivo biological efficacy.

The synthesis of the two analogues (**5** and **6**) was conducted as illustrated in Scheme 1. Treatment of γ-butyrolactone (**7**) with MeNHOMe and Me_2AlCl afforded the corresponding Weinreb amide,[7] which was converted to a keto-alcohol (**8**) with pentylmagnesium bromide in 78% yield for two steps. Swern oxidation of **8** followed by Wittig reaction with (carbethoxymethylene)triphenylphosphorane provided an α,β-unsaturated ester (**10**) in 90% yield for two steps. Next, the

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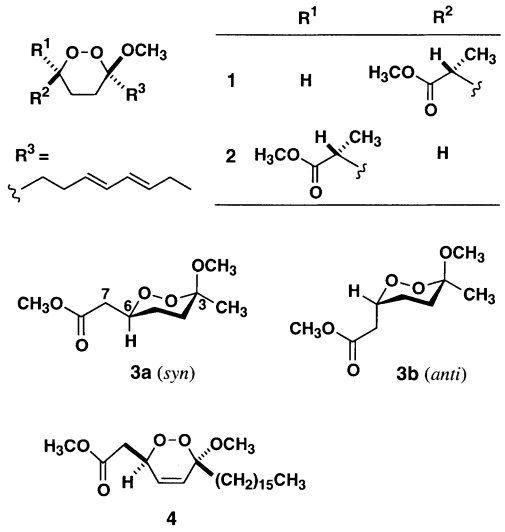


Figure 1.

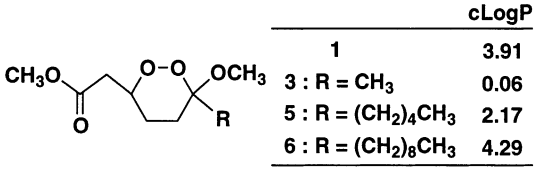


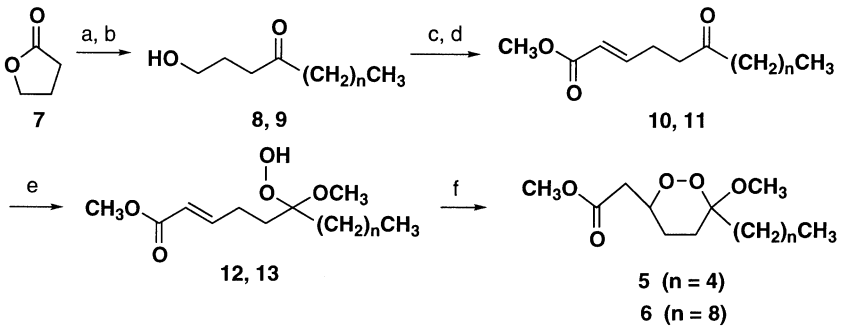
Figure 2.

unsaturated ester **10** was submitted to $Sc(OTf)_3$-mediated peroxyhemiacetalization[3] to afford **12** in 68% yield. Contrary to our expectation, the intramolecular Michael addition[3] of **12** with Et_2NH in $(CF_3)_2CHOH$ caused considerable reduction of the yield of the desired 1,2-dioxane **5** (14%) unlike the preparation of **3**.

A significant amount of formation of an epoxyketone **14** in comparison with the preparation[3] for **3** led us to presume that the larger steric hindrance of the pentyl group in **5** would bring about a degradation of **5** in $(CF_3)_2CHOH$ with high acidity (Table 1, entry 1). In fact, treatment of **5** in $(CF_3)_2CHOH$ at room temperature gave a mixture of **5** and **10** in a ratio of 1:1. On the other hand, compound **5** was completely stable in CF_3CH_2OH. Thus, the reaction conditions from **12** to **5** were investigated using CF_3CH_2OH as a reaction solvent. As shown in entry 2, the replacement of $(CF_3)_2CHOH$ with CF_3CH_2OH resulted in increase of the yield of **5**. Reduction of the concentrations of **12**, Et_2NH, and reaction temperature stimulated the generation of **5**. Eventually, the condition illustrated in entry 6 afforded **5** (**5a**:**5b** = 8.3:1) in moderate yield (62%). By application of this condition, a nonyl congener **6** (**6a**:**6b** = 6:1) was also synthesized from **13** in a similar yield as shown in entry 7.

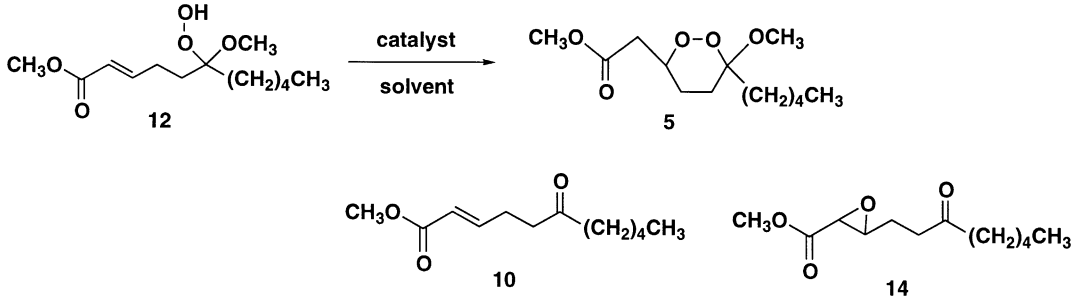
First of all, we assessed biological potency of the analogues without separating the diastereomeric mixtures. Table 2 summarizes the growth inhibitory activity against *P. falciparum* and the selective toxicity index between parasite and cells of host-animals, human epidermoid carcinoma KB 3-1.[8] While the two designed analogues (**5** and **6**) showed similar potent growth inhibitory activities against *P. falciparum* comparable to the methyl esters (**1** and **2**) of peroxyplakoric acids A_3 and B_3, the nonyl congener **6** showed about 10-fold stronger cytotoxic effect against KB 3-1 cells than **5**. With respect to the pentyl analogue **5**, the reduction of cytotoxicity resulted in a higher selective toxicity score (360) relative to those of **1** and **2**.

Next, the anti-malarial potency of **5** and **6** was evaluated after separation of the respective diastereomeric isomers. In order to carry out unambiguous elucidation of the relative stereostructures of both isomers, the methyl congener **3**, which showed a simple signal pattern in the ^1H NMR spectrum, was analyzed. The coupling constants and NOE correlations revealed the methoxyl groups on C-3 to adopt axial orientation in both isomers. In the NOESY spectrum of **3a**, the following pairs of protons, Hax-4 and Hax-6, and 3-OCH_3 and Hax-5, were correlated to each other. On the contrary, the NOESY spectrum of **3b** showed the correlations among Hax-4 and H-7b, Heq-5 and H-7a, Heq-4 and Hax-5, and Hax-4 and 3-CH_3. Therefore, the former **3a** and the latter **3b** were deduced to be the *syn*- and the *anti*-dioxane, respectively.[9] Comparison of the ^1H NMR data between **3a** and **3b** disclosed the conclusive difference that the signals ascribable to H_2-7 in **3b** were resonated at lower field owing to deshielding effect from oxygen atoms in the dioxane ring as compared with those in **3a**. By application of this physicochemical property, *syn*- (**5a** and **6a**) and *anti*-isomers (**5b** and **6b**) were completely distinguished after HPLC separation.[10]



Scheme 1. Synthesis of the designed analogues (**5** and **6**). Reagents and conditions: (a) MeNHOMe, Me_2AlCl; (b) BrMg-R, [R = $(CH_2)_nCH_3$], two steps 78% for **8** (n = 4), 75% for **9** (n = 8); (c) $(COCl)_2$, DMSO, Et_3N; (d) $Ph_3P{=}CHCOOCH_3$, CH_2Cl_2, two steps 90% for **10** (n = 4), 89% for **11** (n = 8); (e) $H_2O_2{\cdot}H_2NCONH_2$, $Sc(OTf)_3$, MeOH, 68% for **12** (n = 4), 67% for **13** (n = 8); (f) Et_2NH, CF_3CH_2OH, 62% for **5** (n = 4), 60% for **6** (n = 8).

Table 1. Investigation on Michael addition of the peroxyhemiacetal



Entry	Temperature	Time	Concn of **12** (M)	Concn of base (M)	Solvent	Yield (%)[a]			
						12	**5**	**10**	**14**
1	rt	8 h	0.16	0.01	$(CF_3)_2CHOH$	0	14	29	57
2	rt	8 h	0.16	0.01	CF_3CH_2OH	0	22	47	31
3	0	2 day	0.16	0.01	CF_3CH_2OH	0	33	45	22
4	rt	8 h	0.016	0.001	CF_3CH_2OH	15	51	8	26
5	rt	8 h	0.016	0.005	CF_3CH_2OH	0	55	19	26
6	0	2 day	0.016	0.005	CF_3CH_2OH	0	62	18	20
7[b]	0	2 day	0.016	0.005	CF_3CH_2OH	0	60	19	21

[a]In all reactions, the peroxyhemiacetals (**12** and **13**) gave the three products exclusively. The yields were estimated by ^1H NMR spectra after concentration of reaction mixtures.
[b]The nonyl congener **13** was used in the reaction. The yields of the corresponding congeners were given.

As designated in Table 2, the *syn*-isomers exhibited higher selective toxicity scores than the *anti*-ones in either case. Nevertheless, this purification little affected the anti-malarial potency of **5** and **6**. Finally, participation of the absolute configurations in the biological activity was examined in the case of the most potent analogue **5a**. The assessment of the biological property after chiral column separation[11] clarified that the asymmetric centers of the peroxides are independent of their anti-malarial potency.

In summary, 6-carbomethoxymethyl-3-methoxy-3-pentyl-1,2-dioxane (**5**), which was accessibly synthesized by utilizing the spongean peroxides as a scaffold, was shown to exhibit potent anti-malarial activity with selective toxicity. Comparing the anti-malarial potency among **1**, **2**, and **5**, not only the methyl residues on the asymmetric center but also the diene portions in the side chains of **1** and **2** were shown to participate in the biological activity slightly. Investigation of in vivo anti-malarial effect of **5** is in progress in our laboratory.

Table 2. Anti-malarial activity and cytotoxicity of 1,2-dioxanes

Compd	IC_{50} (μM)		Selective toxicity
	P. falciparum	KB 3-1	
1	0.15	21	140
2	0.12	28	230
3	>2.4	>0.49	
4	>0.24	0.63	<2.6
5	0.12	43	360
5a (*syn*)	0.15	53	350
5b (*anti*)	0.15	23	150
6	0.14	4.4	31
6a (*syn*)	0.17	4.4	26
6b (*anti*)	0.26	2.2	8
(+)-**5a**	0.22	77	350
(−)-**5a**	0.22	82	370

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8. A strain of *P. falciparum* (FCR3, cycloguanil-resistant from Gambia) was used in sensitivity testing. After synchronization by the sorbitol treatment, a 50 μL of parasite culture at ring stage (0.55% parasitemia and 2% hematocrit) was added to each well in 96-well microculture plates. The test samples were dissolved in DMSO and diluted to the appropriate concentration using complete medium, then a 50 μL of each sample

solution was inoculated. The final concentration of DMSO in the culture is 1%. After incubation at 37 °C for 48 h, the proliferation of *P. falciparum* was assessed by Giemsa-stained smear by observing 10,000 erythrocytes per one thin blood film. Quinine was used as a reference anti-malarial. In this anti-malarial assay, quinine inhibited the proliferation of *P. falciparum* in a concentration-dependent manner with IC_{50} of 40 ng/mL and IC_{90} of 90 ng/mL. Cytotoxic potency was evaluated by colorimetric MTT assay, in which mitomycin C used as a positive control showed the IC_{50} of 0.1 μg/mL.

9. **3a**: colorless oil. IR υ_{max} (KBr) cm^{-1}: 1745. ^1H NMR (500 MHz, $CDCl_3$) δ 1.19 (3H, s, 3-CH_3), 1.55 (1H, dddd, J = 15.3, 4.9, 4.3, 2.4 Hz, Heq-5), 1.67 (1H, ddd, J = 16.5, 12.2, 4.3 Hz, Hax-4), 1.75 (1H, dddd, J = 15.3, 12.2, 11.0, 4.3 Hz, Hax-5), 1.83 (1H, ddd, J = 16.5, 4.9, 4.3 Hz, Heq-4), 2.32 (1H, dd, J = 15.9, 5.5 Hz, Ha-7), 2.44 (1H, dd, J = 15.9, 7.9 Hz, Hb-7), 3.24 (3H, s, 3-OCH_3), 3.63 (3H, s, CO_2CH_3), 4.43 (1H, dddd, J = 11.0, 7.9, 5.5, 2.4 Hz, H-6). FAB-MS m/z 227 $(M+Na)^+$. FAB-HR MS m/z calcd for $C_9H_{16}O_5Na$: 227.2117, found: 227.2101; **3b**: colorless oil. IR υ_{max} (KBr) cm^{-1}: 1745. ^1H NMR (500 MHz, $CDCl_3$) δ 1.22 (3H, s, 3-CH_3), 1.37 (1H, m, Hax-5), 1.69 (1H, d, J = 6.1 Hz, Hax-4), 1.70 (1H, dd, J = 5.5, 1.8 Hz, Heq-4), 2.20 (1H, dddd, J = 14.0, 6.7, 6.1, 5.5 Hz, Heq-5), 2.54 (1H, dd, J = 15.3, 6.1 Hz, Ha-7), 2.93 (1H, dd, J = 15.3, 7.9 Hz, Hb-7), 3.27 (3H, s, 3-OCH_3), 3.64 (3H, s, CO_2CH_3), 4.46 (1H, dddd, J = 7.9, 6.7, 6.1, 5.5 Hz, H-6). FAB-MS m/z 227 $(M+Na)^+$. FAB-HR MS m/z calcd for $C_9H_{16}O_5Na$: 227.2117, found: 227.2101.

10. **5a**: colorless oil. IR υ_{max} (KBr) cm^{-1}: 1745. ^1H NMR (500 MHz, $CDCl_3$) δ 0.89 (3H, t, J = 7.3 Hz, CH_3), 1.24–1.40 (6H, m), 1.58–1.70 (3H, m), 1.75–1.94 (3H, m), 2.38 (1H, dd, J = 15.9, 5.5 Hz, Ha-7), 2.51 (1H, dd, J = 15.9, 7.3 Hz, Hb-7), 3.26 (3H, s, 3-OCH_3), 3.70 (3H, s, CO_2CH_3), 4.49 (1H, m, H-6). FAB-MS m/z 283 $(M+Na)^+$. FAB-HR MS m/z calcd for $C_{13}H_{24}O_5Na$: 283.1526, found: 283.1521. **5b**: colorless oil. IR υ_{max} (KBr) cm^{-1}: 1745. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.90 (3H, t, J = 7.3 Hz, CH_3), 1.25–1.50 (8H, m), 1.50–1.80 (3H, m), 2.26 (1H, m), 2.62 (1H, dd, J = 15.9, 6.1 Hz, Ha-7), 3.02 (1H, dd, J = 15.9, 7.9 Hz, Hb-7), 3.29 (3H, s, 3-OCH_3), 3.71 (3H, s, CO_2CH_3), 4.54 (1H, m, H-6). FAB-MS m/z 283 $(M+Na)^+$. FAB-HR MS m/z calcd for $C_{13}H_{24}O_5Na$: 283.1526, found 283.1521. **6a**: colorless oil. IR υ_{max} (KBr) cm^{-1}: 1745. ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t, J = 6.7 Hz, CH_3), 1.20–1.38 (15H, m), 1.58–1.70 (2H, m), 1.75–1.93 (3H, m), 2.38 (1H, dd, J = 15.9, 5.5 Hz, Ha-7), 2.51 (1H, dd, J = 15.9, 7.3 Hz, Hb-7), 3.26 (3H, s, 3-OCH_3), 3.70 (3H, s, CO_2CH_3), 4.49 (1H, m, H-6). FAB-MS m/z 339 $(M+Na)^+$. FAB-HR MS m/z calcd for $C_{17}H_{32}O_5Na$: 339.2156, found: 339.2147. **6b**: colorless oil. IR υ_{max} (KBr) cm^{-1}: 1745. ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t-like, J = ca. 6.5 Hz, CH_3), 1.19–1.38 (14H, m), 1.38–1.49 (2H, m), 1.50–1.80 (3H, m), 2.25 (1H, m), 2.62 (1H, dd, J = 15.9, 6.0 Hz, Ha-7), 3.02 (1H, dd, J = 15.9, 7.9 Hz, Hb-7), 3.29 (3H, s, 3-OCH_3), 3.71 (3H, s, CO_2CH_3), 4.54 (1H, m, H-6). FAB-MS m/z 339 $(M+Na)^+$. FAB-HR MS m/z calcd for $C_{17}H_{32}O_5Na$: 339.2156, found: 339.2147.

11. Enatiomeric mixture of **5a** was separated with Chiralcel OJ column (4.6 i.d.×250 mm, Daicel Chemicals Co. Ltd.) using *n*-hexane/AcOEt (30:1) as an eluent to furnish (+)-**5a** {$[\alpha]_D$ +227° (*c* 0.1, $CHCl_3$)} and (−)-**5a** {$[\alpha]_D$ −222° (*c* 0.1, $CHCl_3$)}. The absolute configurations of both have never elucidated because of their similar anti-malarial potency.