ANTICOCCIDIAL ACTIVITY OF NOVEL SEMI-SYNTHETIC ANALOGUES OF DEOXYFRENOLICIN AND FRENOLICIN B (PART II)

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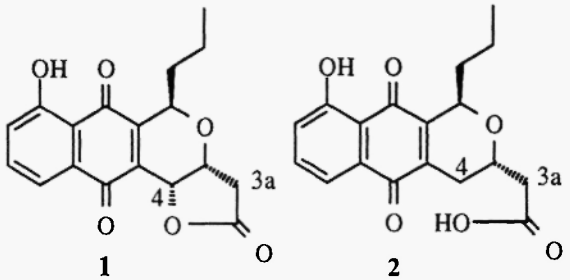
Abstract: Semi-synthetic C-3a and C-4 substituted analogues of the naphthopyranquinones, frenolicin B **1**, and deoxyfrenolicin **2**, have been produced and their biological activity as anticoccidial agents investigated *in vivo*.

INTRODUCTION

In a previous paper[1], we described the synthesis and *in vivo* anticoccidial activities of some semi-synthetic analogues of the naphthopyranquinone, frenolicin B **1** in which modifications had been carried out in the benzofused ring of the naphthoquinone. Apart from phenolic pro-drugs derived from **1** these modifications led to less potent anticoccidial agents.

It has previously been reported that deoxyfrenolicin **2** is virtually devoid of *in vivo* anticoccidial activity.[2] In this paper we describe the synthesis and anticoccidial activity of some analogues of **1** and **2** in which modifications have been made at the C-4 and C-3a positions.



RESULTS AND DISCUSSION

The initial work in this area focused on chemistry at the C-4 position. For the purpose of direct comparison within the C-4 substituted series the methyl ester **3** was synthesised by treatment of **2** with methanol and catalytic pTSA (100%) (Figure 1).

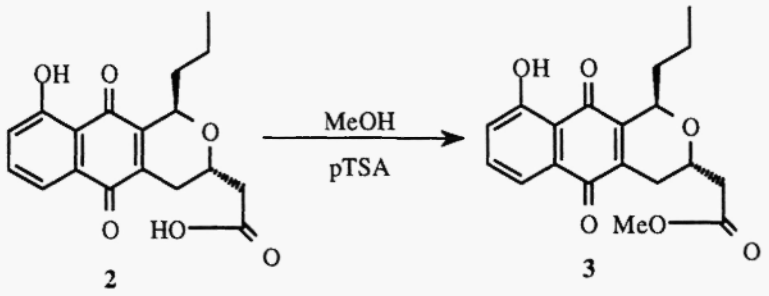


Figure 1

Refluxing of a solution of **1** in methanol treated with a catalytic amount of concentrated hydrochloric acid led to the formation of **4** as a 5:1 *cis*:*trans* diastereomeric mixture (41%) (Figure 2). This analogue presumably arises from lactone ring methanolysis followed by the trapping of a quinone methide type intermediate with methanol, as stereochemical integrity is not conserved in the reaction. The attack of methanol onto the quinone methide intermediate preferentially occurs from the opposite, less sterically hindered face to the n-propyl side chain. In an analogous fashion treatment of **1** with 2N ethanolic hydrogen chloride led to the formation of **5** as a 4:1 *cis*:*trans* mixture (37%) (Figure 3).[3]

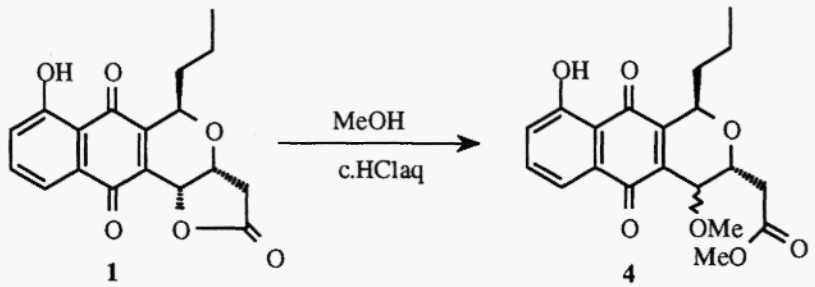


Figure 2

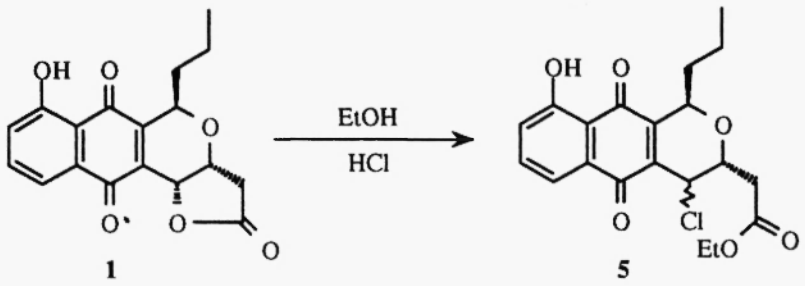


Figure 3

Treatment of **1** with aqueous sodium hydroxide at room temperature followed by careful reacidification led to the formation of **6** as a single diastereomer (53%).[3] Subsequent treatment of **6** with trimethylsilyldiazomethane gave the corresponding methyl ester **7** (69%). Conversion of **7** to the C-4 acetoxy substituted analogue **8** was completed by reaction of **7** with one equivalent of acetic anhydride in pyridine (47%) (Figure 4).

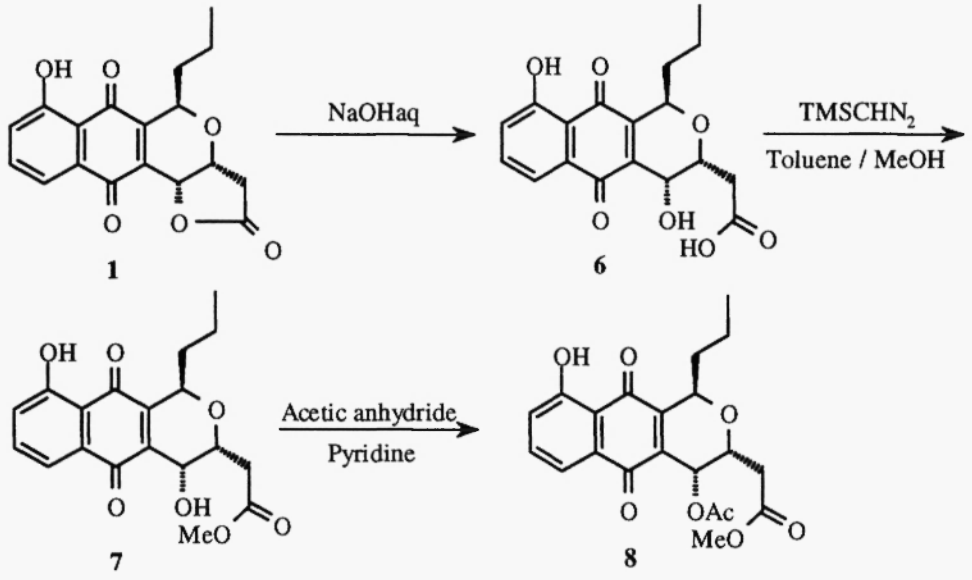
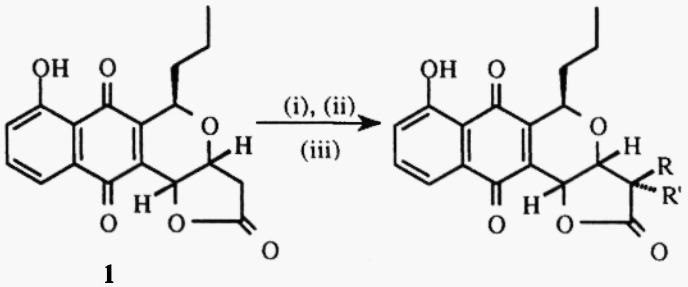


Figure 4

Chemistry to probe the effect of substituents at C-3a was facilitated by the discovery that treatment of **1** with 2.5 equivalents of lithium bis(trimethylsilyl)amide in tetrahydrofuran at -78°C gave the reasonably stable dianion of **1**. Subsequent treatment of this dianion with various electrophiles followed by acidic work up gave rise to compounds which were stereoselectively alkylated at the C-3a position. In most cases a single diastereomeric product was isolated in poor yield (5-15%) in which the electrophile had reacted on the least hindered face of the lactone enolate.[7] In the case of more reactive electrophiles, such as methoxymethyl bromide, trace amounts of disubstituted products at the C-3a position were also isolated (Figure 5).



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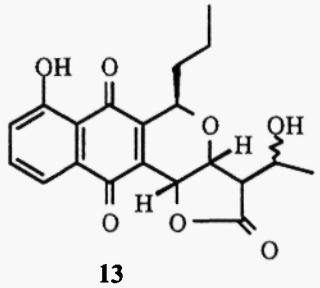
(i) LiHMDS, THF, -78°C
(ii) Electrophile
(iii) 2N HCl(aq)

Electrophile	R	R'	
MeI	Me	H	**9**
$PhCH_2Br$	CH_2Ph	H	**10**
$MeOCH_2Br$	CH_2OMe	H	**11**
$MeOCH_2Br$	CH_2OMe	CH_2OMe	**12**

Figure 5

Aldol reaction of the dianion derived from **1** with acetaldehyde gave a 2:1 diastereomeric mixture about the newly formed hydroxyl centre of the β-hydroxy ketone **13**.



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The anticoccidial activities of some of the analogues described herein were measured using a standard 7 day *in vivo* chick model utilising a polyether ionophore resistant field isolate of *Eimeria tenella*.[4] The results of these tests are shown below (Table 1) where the effective dose is defined as the in feed dose (ppm) required to give a mean (n=5) reduction in cecal lesion score of >80% with respect to non-medicated controls.

Table 1

Compound	Effective Dose (ppm)	Compound	Effective Dose (ppm)
1	25-50	**2**	100
3	>100	**4**	>50
6	>50	**7**	>50
8	>50	**11**	>50
13	>100		

The results indicated that the modifications that were carried out at C-3a and C-4 led to a reduction or loss of *in vivo* anticoccidial activity.

The reduced activity demonstrated by **2** was assumed to arise from slow conversion to **1** *in vivo* although we were able to demonstrate this conversion occurred to differing extents *in vitro* in organic solvents and aqueous buffers of varying pH under aerobic conditions. In an attempt to pursue this hypothesis further the methyl ester **3** was also examined under similar conditions to **2** *in vitro*. In the case of **3**, no conversion to **1** was apparent on the timescale in which conversion of **2** to **1** occurred and this was also demonstrated by the lack of activity shown by for **3** *in vivo*.

The lack of activity demonstrated by **3** led us to believe that a leaving group may be required at C-4 to facilitate the formation of a reactive quinone methide which may be responsible for the anticoccidial efficacy.[5,6] Compound **8** contains a acetoxy leaving group at C-4 which we envisaged would have similar leaving group potential to the lactone present in **1**. Compound **8** however, failed to show any activity *in vivo* at 50ppm in feed whereas **1** showed consistent activity at this dose.

This finding led us to believe that a tethered leaving group at C-4 was required as in the lactone present in **1** and so analogues in which C-3a was substituted were investigated. The monosubstituted analogues **11** and **13** failed to show any activity at 50 and 100 ppm respectively *in vivo*. The precise reasons for the lack of activity in these analogues is unknown. However, it may be hypothesised that the C-3a substituent is affecting the leaving group potential of the lactone ring.

CONCLUSION

Several C-3a and C-4 substituted analogues of deoxyfrenolicin and frenolicin B have been synthesised and their anticoccidial efficacy measured *in vivo*. These analogues have been shown to be less potent than frenolicin B indicating that the C-3a and C-4 positions of the naphthopyranquinone nucleus have limited potential for the discovery of a more potent anticoccidial agent from this class.

ACKNOWLEDGEMENTS

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7) Typical procedure for the preparation of **10**:
To a stirred solution of LiHMDS (1.0M in hexanes) (1.8 ml, 1.8 mmol) in THF (1 ml) under nitrogen atmosphere at -78°C was added a solution of **1** (200mg, 0.6 mmol) in THF (2 ml) dropwise. The reaction mixture turned dark red immediately and was stirred for a further 30 min. at -78°C. Benzyl bromide (0.11 ml, 0.9 mmol) was then added dropwise and the reaction mixture stirred for a further 30 min. at -78°C. At this point the reaction was warmed to room temperature and water (2 ml) added followed by aqueous 2N HCl (2 ml). The resulting mixture was extracted into dichloromethane (3 x 10 ml) and the organic layer separated, dried (Na_2SO_4) and solvent removed under reduced pressure. The product was purified by flash column chromatography on silica (2 : 1 hexane : ethyl acetate eluent) to give **10** as a bright yellow powder (10 mg, 4%). ^{1}H NMR ($CDCl_3$) (300MHz): δ 0.85 (3H, t, J = 8Hz), 1.4 (3H, m), 1.65 (1H, m), 2.95 (2H, m), 3.25 (1H, dd, J = 15 and 3Hz), 4.25 (1H, d, J = 3Hz), 4.85 (1H, dd, J = 15 and 3Hz), 4.95 (1H, d, J = 3Hz), 7.3 (6H, m), 7.7 (2H, m), 11.8 (1H, s); *m/z* (thermospray): 436 (MNH_4^+).

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