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Synthesis of a Structurally Constrained Endoperoxide having Antimalarial Activity from α-Santonin

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ABSTRACT

α-Santonin 3 **was successfully converted into a biologically active compound** 5b **containing an endoperoxide group through a photo-oxygenation approach as a single isomer. It was found that the singlet oxygen afforded the isomer produced by attack from the sterically-hindered face of cyclohexadiene derivative** 4**. Evidence to this end is presented based on NOE results and the products formed in the photo-oxygenation reaction, as well as the *in vitro* testing of** 5b **for antimalarial activity.**

KEYWORDS

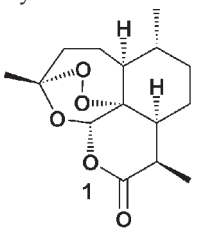
Santonin, endoperoxide, photo-oxygenation, singlet oxygen.

1. Introduction

Among the many and varied maladies that afflict humankind, malaria appears to be making a particularly strong comeback. The resistance that newer strains of the protozoa *Plasmodium falciparum,* and *P. vivax* in particular, show against commonly used drugs has made it imperative that new methods of combating this scourge be sought. This is particularly the case when one considers that the World Health Organisation[1] estimates that 300–500 million new cases of malaria are reported annually, mostly in sub-Saharan Africa.[2] A million people die annually from the disease.

At this time, the only class of compounds against which the malaria parasite has shown no resistance are those based on the natural endoperoxide-containing compound artemisinin (qinghaosu) **1**, a compound isolated from the Chinese herb *Artemisia annua.*[3,4] Endoperoxide **1** is currently used in cases of severe malarial infection by *P. falciparum* and *P. vivax.*[4,5]

Similar activity has been noted using yingzhaosu A **2**, isolated from the Chinese herb *Artabotrys uncinatums.*[6] A drawback associated with **1** is its pronounced neurotoxicity that has been demonstrated *in vitro,* although such activity *in vivo* in humans has yet to be demonstrated.[7] The activity, and by extension the neurotoxicity, of **1** and similar endoperoxides has until recently been associated with the fragmentation of the endoperoxides in the presence of ferrous ions in the food vacuole of the parasite, affording *O*- and *C*-centred radicals.[3,5] Recently, this mode of action was proved incorrect by Krishna and Eckstein-Ludwig,[8] who found that the critical calcium-pumping enzyme PfATP6 was the specific target of the artemisinin family of compounds in the presence of ferrous ions. This discovery makes a targeted approach to developing endoperoxides as antimalarial drugs a reality.



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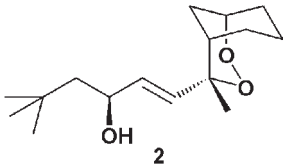

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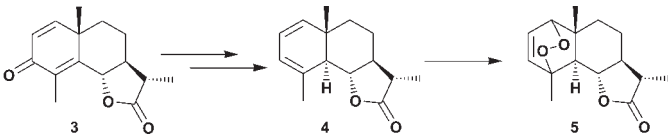
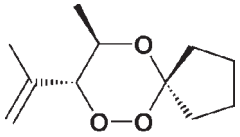
These facts prompted us to begin to develop the methodology to prepare endoperoxides as well as structure-activity data with regard to their activity against malaria. We used a commercially available sesquiterpenoid, α-santonin **3**, a major metabolite of *Artemisia maritime,* as the scaffold on which to build an endoperoxide. This was done by converting **3** into cyclohexadiene **4** using known procedures,[9,10] and then treating this compound with singlet oxygen to afford **5**. This provided a suitably rigid platform of defined stereochemistry on which to begin elaborating our search for active endoperoxides as antimalarials.



3 4 5

Our rationale here was based on the report[11] that simple 1,2,4-trioxane systems such as **6** showed significant antimalarial activity, having inhibitory values against *P. falciparum* of 178 ng mL^{-1} as a racemate – about 90 ng mL^{-1} for the more active isomer as an estimate (*cf.* chloroquin, 65 ng mL^{-1}; and artemisinin, 1.2 ng mL^{-1}). This implied that we could possibly expect some activity from endoperoxide **5**, the techniques developed for the preparation of which could be applied to the synthesis of other materials of similar or improved activity.



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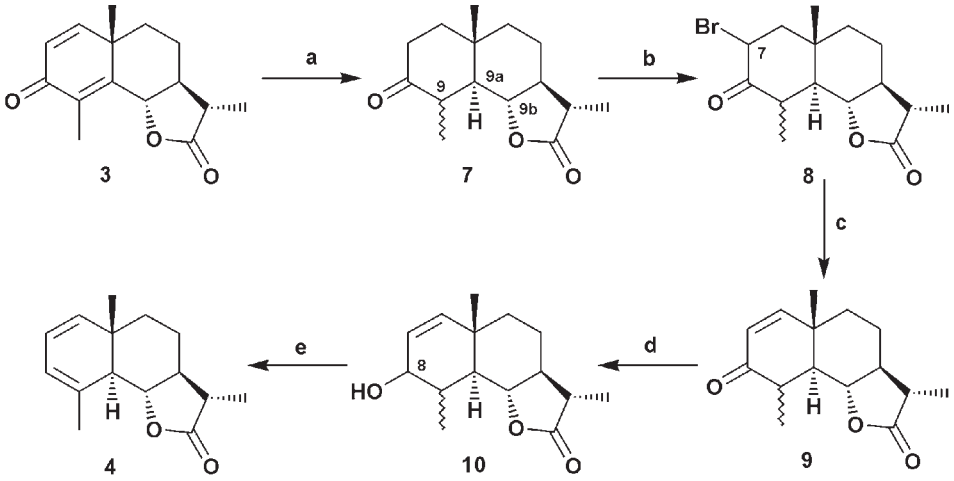
2. Results

2.1. Preparation of Cyclohexadiene Derivative 4

The initial steps for the preparation of cyclohexadiene derivative **4** from **3** (Scheme 1) used methodology developed by Blay *et al.*[9,10]

The sequence involved the reduction of the 1,4-diene-3-one system in **3** to form saturated cyclohexanone **5**. Unlike the case of



Scheme 1
(**a**) 5% Pd/c, HCl, 1 atm H_2 (g); (**b**) Br_2; (**c**)LiI, K_2CO_3; (**d**) $LaCl_3.7H_2O$, $NaBH_4$; (**e**) i) $POCl_3$, 2,6-lutidine; ii) LiI, K_2CO_3

Blay *et al.*, we only required H-9a to be set, the planarity of diene **4** removing any stereochemical information encoded by H-9. Blay *et al.* claimed 95:5 H-9/H-9a *cis*:*trans* selectivity in the hydrogenation of **3** to *trans*-fused ketone **7** using NaTaH as a proton donor in the reduction medium. In our hands, reduction in ethyl alcohol without an added proton donor afforded a mixture of all four isomers in about 90% yield. In the presence of a drop of concentrated hydrochloric acid, however, the reduction in ethanol afforded a 3:2 H-9/H-9a *cis*:*trans* ratio of **7** in essentially quantitative yield. That both species were *trans*-fused across the AB ring was shown by a 10–11 Hz coupling constant in the H-9b signals of each epimer at δ 3.92 ppm and δ 4.30 ppm, respectively, in the ^{1}H NMR spectrum of the mixture.

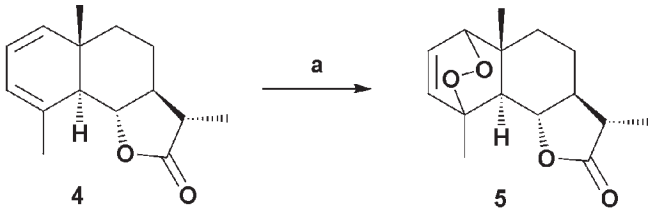
Selective bromination at the least hindered position adjacent to the carbonyl functionality of mixture **7** produced α-bromo ketone **8** as an epimeric mixture at H-9 in quantitative yield. The stereochemistry at C-7 was assigned based on published results,[10] and is consistent with the NMR spectra that show the presence of two isomers, i.e. that bromine has been stereoselectively delivered, probably from the lower face due to steric constraints. Dehydrobromination under basic conditions afforded a 3:1 mixture of epimeric enones **9** in 55% yield. Sodium borohydride-mediated reduction of **9** in the presence of lanthanum chloride hydrate proceeded stereoselectively according to ^{1}H NMR spectroscopy, affording a mixture of two allylic alcohols **10**.[12]

Finally, derivatization of allylic alcohol mixture **10** to a chloride, followed by base-mediated elimination afforded the 1,3-cyclohexadiene derivative **4** in 30–40% yield. The product was isolated as a single isomer, according to ^{1}H NMR spectroscopy, indicating that the initial stereochemistry at the bridgehead had been set, as desired. We were now in a position to proceed with the formation of our endoperoxide derivative.

2.2. Formation of Biologically Active Endoperoxide 5

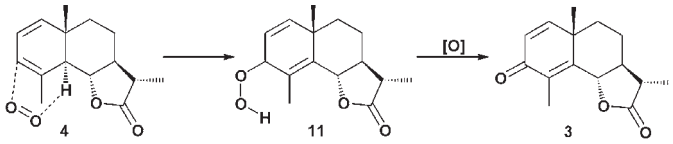
We envisaged using a photochemically prepared singlet oxygen methodology to introduce the peroxide moiety (Scheme 2). Using 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine[13] as the photosensitizer, diene **4** was exposed to high-intensity light under a stream of oxygen in a quartz photochemical cell for 18 hours. A new product, **5**, was obtained as a single isomer in 20–30% yield.

Interestingly, Huffman[14] reported that photo-oxygenation of diene **4** using eosin failed to generate an endoperoxide, but rather re-generated santonin **3**. Under the conditions mentioned

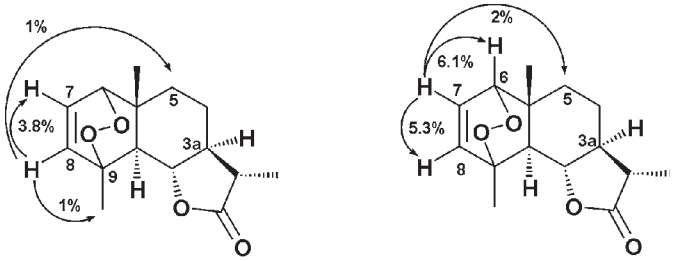


Scheme 2
(**a**) TPP, hν, O_2 (g)

in Scheme 2, ^{1}H NMR spectroscopy of the crude reaction mixture identified about 25% of **3** in the sample along with endoperoxide **5**. This was probably formed through a six-membered transition state involving the C-8:C-9:H-9a system and oxygen, resulting in a species similar to that which would be formed by an ene-type process. Oxidation of the intermediate hydroperoxide **11** under the reaction conditions would afford **3**.

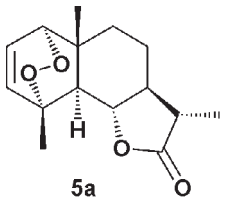


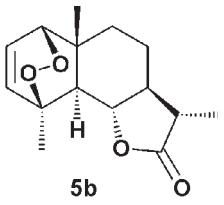
At the outset, we expected that singlet oxygen would have approached from the least hindered face of **4**, thus avoiding the steric influence of the axial methyl group at C-5a. In an attempt to prove that this was the case, NOE difference spectroscopy was undertaken on the two alkene hydrogen atoms individually to determine their relative influences on nearby protons. Bearing in mind that the atoms in question are part of an unsaturated system that will allow rapid relaxation of the protons, paired with the unfavourable orientation of the atoms away from most of the protons in free space, we did not expect strong responses from any affected protons. The responses are summarized as follows.



The significant, although admittedly weak, interaction of alkene proton H-7 with a proton in the 1.43–1.54 ppm region of the proton NMR spectrum was the telling factor. This region accounts for three protons in the molecule, namely H-3a and the C-5 protons, which were not clearly resolved on the 400MHz spectrum. By inspection, it is clear that the NOE interaction is likely due to an H-7 to H-5 interaction, as H-3a is too far away for a significant through-space interaction of any kind. Molecular modelling[15] indicated that, for both **5a** and **5b**, one of the protons at C-5 occupies the axial position projecting *below* the plane of the ring system as drawn, while the other occupied the standard equatorial position.

If singlet oxygen had attacked from the least-hindered face of **4**, endoperoxide **5a** would have resulted. This structure has H-7 and H-8 projecting *above* the plane of the ring system. A clear NOE would have been seen between H-7 or H-8 and the axial methyl group at C-5a, which is about 2.93 Å away from H-7 in **5a**. The protons on C-5, on the other hand, are 4.99 Å and 5.24 Å away from H-7 for H-5_{eq} and H-5_{ax}, respectively. In structure **5b**, which typifies the attack of singlet oxygen from the upper face of the ring system, H-7 and H-8 are projecting *below* the plane. Molecular modelling indicated that the distance between H-7 and the protons at C-5 are 3.57 Å and 4.38 Å for H-5_{ax} and H-5_{eq}, respectively, while the distance between H-7 and the axial methyl group at C-5a is about 5.71 Å. This would imply that a through-space interaction between H-7 and H-5_{ax} is likely, while that to the axial methyl group at C-5a is not as preferred. No NOE interaction is observed between either H-7 or H-8 and the axial methyl group at C-5a in either the standard 1-D NOE difference spectra or in a full NOESY spectrum from a 400 MHz instrument. While not proving anything on its own by the absence of an interaction, this fact along with the observed NOE between H-7 and H-5_{ax} suggest that the alkene system is puckered *below* the ring system in order to interact with H-5_{ax}, in turn indicating that *the endoperoxide had formed by attack of singlet oxygen on the more sterically hindered face of cyclobutadiene **4***, affording compound **5b**.





Furthermore, the occurrence of santonin **3** in the reaction mixture adds credence to the orientation of the endoperoxide moiety in **5b**. As has already been mentioned, the formation of santonin **3** noted in the reaction mixture may have formed by a six-membered transition state. If so, the mechanism would probably require that the oxygen approaches from the *lower* face of **4** in order to interact with H-9a in order for the ene reaction to occur. This is not possible from the upper face of **4**. The presence of endoperoxide **5b** to the exclusion of **5a** is thus the only possible reaction product allowed by the approach of oxygen from the *upper* face of **4**. The two possible products hence point to a delicate balance between reactivity of the oxygen, the structure of the diene and the potential reaction pathways open to the two interacting components as a result of these factors. Interestingly, molecular dynamics calculations indicated that **5b** was more than 3 kcal more stable than **5b**, contrary to intuitive deduction.

In order to probe the reactivity of **5b**, the mild reductive method used by Hioki *et. al.*[16] employing sodium borohydride in methanol at 50°C was used in an attempt to open the peroxide selectively. However, the product formed was not diol **12**, but 4-methoxycyclohexanone **13** in 53% yield. ^{1}H and ^{13}C NMR spectroscopy indicate that the product is a single isomer. The exact mechanism of the formation of **13** is uncertain. Interestingly, **13** bears close resemblance to the known antihelmintic sesquiterpenoid from the Asian plant *Artemisia vulgaris,*[14] tauremisin (vulgarin) **14**.

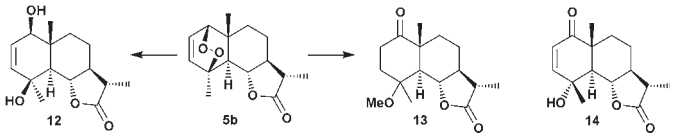


Table 1 Comparison of the antimalarial activity of **1**, **5b**, **6** and chloroquine

Compound	IC_{50} / μg.cm^{-3}	IC_{50} / nM
Artemisinin **1**[17]	2.4×10^{-3}	8.4
Yingzhaosu A **2**[18]	4.6×10^{-3}	17
Chloroquine[17]	11.8×10^{-3}	37
1,2,4-Trioxane **6**[11]	178×10^{-3}	898
Endoperoxide **5b**[17]	4.43	1676

2.3. Antimalarial Activity of Compound 5b

The *in vitro* assay results of the endoperoxide **5b** against *P. falciparum* is summarized with other antimalarial compounds and chloroquine for comparison in Table 1.

The results indicate that, although **5b** is considered 'active' (any compound with IC_{50} <10 μg cm^{-3} is said to be active), it is some three orders of magnitude less active than artemisinin **1**, yingzhaosu A **2** or chloroquine. While this is encouraging, it would seem to indicate that there are severe structural constraints involved in **5b** that prevent either the access of the radicals to the membranes of the parasite, or are involved in some mode of deactivation of the material itself. These questions will be addressed at a later stage.

3. Experimental

General Information

All starting materials used were commercially obtained and used as received from the supplier. Tetrahydrofuran was dried over a sodium/benzophenone ketyl in nitrogen atmosphere, while toluene, 2,6-lutidine and pyridine were dried over sodium using a calcium chloride drying tube. Dichloromethane, *N,N*-dimethylformamide and dichloromethane were distilled from calcium hydride and stored over fresh CaH_2 in sealed bottles before use.

Column chromatography was performed using Merck Kiesel gel 60 (particle size 0.040–0.063 mm), while thin-layer chromatography was done on Merck aluminium-supported silica gel 60 F_{254}. Preparative thin-layer chromatography was performed on Merck aluminium-supported silica gel 60 WF_{254} 20 cm × 20 cm sheets.

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer operating at 200 MHz, or a Varian Unity Plus 400 MHz spectrometer for 2D experiments. Chemical shift data is recorded in ppm, and coupling constants are quoted in Hertz. An asterisk denotes assumed stereochemistry. Optical rotations were measured on a Bellingham and Stanley Ltd ADP 220 polarimeter using the sodium D line, in units of 10^{-1} deg cm^{-2} g^{-1}.

Extractions were followed by drying with magnesium sulphate, and concentration refers to evaporation under reduced pressure on a rotary evaporator.

*(3S,3aS,5aS,9S,9aR,9bS)-3,5a,9-Trimethyloctahydronaphtho[1,2-b] furan-2,8-dione and its 9*R *Epimer* ***7***

α-Santonin **3** (4.50 g, 18.3 mmol) was dissolved in ethyl alcohol (96%, 50 cm^3) to which 32% hydrochloric acid (0.5 cm^3, 4.4 mmol) had been added. 5% Palladium on carbon (896 mg) was added, and the mixture purged with hydrogen gas delivered by a balloon. The mixture was then left for 18 h at room temperature under one atmosphere of hydrogen gas. The catalyst was then filtered off, and the green solution concentrated to a brown oil. Residual catalyst and the acid were removed over a silica plug to afford a white crystalline solid, (3*S*,3a*S*,5a*S*,9*S*,9a*R*, 9b*S*)-3,5a,9-Trimethyloctahydronaphtho[1,2-*b*]furan-2,8-dione and its 9*R* epimer **7** as a 3:2 mixture of isomers (4.54 g, quant.); R_f 0.77 [1:1 (v/v) ethyl acetate : *c*-hexane]. δ_H (200 MHz; $CDCl_3$) 4.34 (0.4H, dd, 9*R* isomer H-9b, *J* 11.0 and 4.5), 3.92 (0.6H, br t, 9*S* isomer H-9b, *J* 10.3), 2.65–1.45 (12H, 8 × m, H-3, H-3a, H-4, H-5, H-6, H-7, H-9 and H-9a for 9*S* isomer and 9*R* isomer), 1.29 (3H, s, C-5a CH_3), 1.23 (3H, d, C-9 CH_3, *J* 7.1) and 1.19 (3H, d, C-3 CH_3, *J* 6.8).

*(3S,3aS,5aS,7R,9S,9aR,9bS)-7-Bromo-3,5a,9-trimethyloctahydro naphtho[1,2-b] furan-2,8-dione and its 9*R *Epimer* ***8***

The mixture of (3*S*,3a*S*,5a*S*,9*S*,9a*R*,9b*S*)-3,5a,9,9a-tetramethyl-octahydronaphtho[1,2-*b*]furan-2,8-dione and its 9*R* epimer **7** (4.54 g, 18.1 mmol) was dissolved in chloroform (178 cm^3). Bromine (1.0 cm^3, 19.6 mmol) in tetrachloromethane (178 cm^3) was then added drop wise to the stirred solution over 3 h at 0°C. The red-brown solution was extracted with saturated aqueous sodium metabisulfite solution, followed by saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried and concentrated to a brown gum, which crystallized in most cases. A mixture of (3*S*,3a*S*,5a*S*,9*S*,9a*R*,9b*S*)-3,5a,9a-trimethyloctahydronaphtho[1,2-*b*]furan-2,8-dione and its 9*R* epimer **8** as a 3:2 mixture of isomers and used as a crude compound; R_f 0.46 [1:2 (v/v) ethyl acetate : hexane]. δ_H (200 MHz; $CDCl_3$) 4.85 (1H, dd, H-7, *J* 13.5 and 6.0), 4.32 (0.4H, dd, 9*R* isomer H-9b, *J* 11.2 and 4.6), 3.91 (0.6H, br t, 9*S* isomer H-9b, *J* 10.3), 2.85–2.62 (1H, m, H-9 for 9*S* isomer and 9*R* isomer), 2.58–1.95, 1.94–1.82, 1.80–1.39 (9H, 3 × m, H-3, H-3a, H-4, H-5, H-6 and H-9a for 9*S* isomer and 9*R* isomer) and 1.38-1.12 (9H, m, C-3 CH_3, C-5a CH_3 and C-9 CH_3 for 9*S* isomer and 9*R* isomer).

*Mixture of (3S,3aS,5aS,9S,9a*R*,9bS)-3,5a,9-Trimethyl-3a,5,5a,9,9a, 9b-hexahydro-3*H*,4*H*-naphtho[1,2-b]furan-2,8-dione and its 9*R *Epimer* ***9***

The mixture of (3*S*,3a*S*,5a*S*,7*R*,9*S*,9a*R*,9b*S*)-7-bromo-3,5a,9-trimethyloctahydro-naphtho[1,2-*b*]*furan-2,8-dione* and its 9*R* epimer **8** (assumed 18.1 mmol from the previous experiment) was dissolved in dry *N,N*-dimethylformamide (178 cm^3). Lithium iodide (11.4 g, 85.2 mmol) and anhydrous potassium carbonate (6.79 g, 49.1 mmol) were then added, and the heated at 120°C for 7 h total. After cooling, the solution was concentrated *in vacuo,* partitioned between 100 cm^3 saturated aqueous sodium hydrogen carbonate solution and 100 cm^3 ethyl acetate. The organic layer was dried and concentrated to a brown residue. Purification by column chromatography using a 1:4-1:2 (v/v) ethyl acetate: *c*-hexane as eluent afforded a yellow-orange oil, *(3S,3aS,5aS,9S,9aR,9bS)-3,5a,9-trimethyl-3a,5,5a,9,9a-hexahydro-3H,4H-naphtho[1,2-b]furan-2,8-dione* and its 9*R* epimer **9** as a 3:1 mixture of isomers (2.72) g, 60% over two steps); R_f 0.39 [1:2 (v/v) ethyl acetate : *c*-hexane]. δ_H (200 MHz; $CDCl_3$) 6.71 (0.25H, d, H-6 for 9*R* isomer, *J* 9.8), 6.53 (0.75H, d, H-6 for 9*S* isomer, *J* 10.2), 5.95 (0.75H, d, H-7 for 9*S* isomer, *J* 10.2), 5.90 (0.25H, d, H-7 for 9*R* isomer, *J* 10.0), 4.38 (0.25H, dd, H-9b for 9*R* isomer, *J* 11.8 and 5.2), 3.91 (0.6H, br t, H-9b for 9*S* isomer, *J* 10.2), 2.67–2.21, 2.11–1.46, 1.94–1.82 and 1.42–1.19 (8H, 4 × m, H-3, H-3a, H-4, H-5, H-9, H-9a and H-9b for 9*S* isomer and 9*R* isomer) and 1.39–1.17 (9H, m, C-3 CH_3, C-5a CH_3 and C-9 CH_3 for 9*S* isomer and 9*R* isomer).

*Two C-8, C-9 Isomers of (3S,3aS,5aS,9aR,9bS)-8-Hydroxy-3,5a,9-trimethyl-3a,4,5,5a,8,9,9a,9b-octahydro-3*H*-naphtho[1,2-b]furan-2-one* ***10***

The mixture of (3*S*,3a*S*,5a*S*,9*S*,9a*R*,9b*S*)-3,5a,9-trimethyl-3a,5,5a,9,9a,9b-hexahydro-3*H*,4*H*-naphtho[1,2-*b*]furan-2,8-dione and its 9*R* epimer **9** (2.72 g, 10.9 mmol) was dissolved in methyl alcohol (100 cm^3, 0.1 M) and cooled to 0°C in an ice bath for 15 minutes. Lanthanum chloride heptahydrate (4.91 g, 13.2 mmol) was added, and the components allowed to mix for 15 min. Sodium borohydride (499 mg, 13.2 mmol) was then added in a single portion, with much effervescence, and the orange solution left to stir for 18 h at room temperature. Saturated aqueous ammonium chloride (50 cm^3) was added, the organics extracted with ethyl acetate and dried, then concentrated to a brown residue. A mixture of *two of the C-8, C-9 isomers of (3S,3aS,5aS,9aR,9bS)-8-hydroxy-3,5a,9-trimethyl-3a,4,5,5a,8,9, 9a,9b-octahydro-3*H*-naphtho[1,2-b]furan-2-one* **10** (2.21 g, 81%) were isolated, and used as-is in the next transformation; R_f 0.48 [1:1 (v/v) ethyl acetate : *c*-hexane]. δ_H (200 MHz; $CDCl_3$) 5.58 (1H, dd, H-7, *J* 10.0 and 2.3), 5.42 (1H, dd, H-6, *J* 9.8 and 2.2), 4.40 (1H, dd, H-9b, *J* 11.4 and 5.1), 3.96–3.72 (1H, br m, H-8), 2.39–2.13, 2.09–1.69, 1.68–1.33 and 1.32–0.83 (8H, 5 × m, H-3, H-3a, H-4, H-5, H-9 and H-9a) and 1.31–1.10 (9H, m, C-3 CH_3, C-5a CH_3 and C-9 CH_3).

*(3S,3aS,5aS,9aR,9bS)-3,5a,9-Trimethyl-3a,4,5,5a,9,9a-hexahydro-3*H*-naphtho[1,2-b]furan-2-one* ***4***

Allylic alcohol mixture **10** (2.21 g, 8.83 mmol) was dissolved in dichloromethane (90 cm^3) and cooled to 0°C in an ice bath for 10 min under nitrogen atmosphere. 2,6-Lutidine (10.5 cm^3) was added, followed by phosphorus oxychloride (1.70 cm^3, 18.2 mmol). The resultant orange solution was stirred for 18 h, while warming gradually to room temperature. Saturated aqueous ammonium chloride (100 cm^3) was added, the organics extracted with dichloromethane and then concentrated to a brown oil. This residue was taken up in *N,N*-dimethylformamide (90 cm^3) and then treated with lithium iodide (7.19 g, 42.3 mmol) and potassium carbonate (2.53 g, 18.3 mmol). The mixture was heated at 70°C in an oil bath for 18h in nitrogen atmosphere. After cooling, the solvent was stripped off, and the residue partitioned between saturated aqueous ammonium chloride and ethyl acetate (30 cm^3 each). The organic layer was dried and concentrated to brown oil. Column chromatography afforded an orange oil, *(3S,3aS,5aS,9aR,9bS)-3,5a,9-trimethyl-3a,4,5,5a,9,9a-hexahydro-3*H*-naphtho[1,2-b]furan-2-one* **4** as a single isomer (618 mg, 30%) along with several unidentifiable products; R_f 0.20 [1:2 (v/v) ethyl acetate : *c*-hexane]. δ_H (200 MHz; $CDCl_3$) 5.85–5.67 (2H, m and s, H-6 and H-7), 5.37 (1H, br d, H-8, *J* 8.1), 4.35 (1H, dd, H-9b, *J* 11.2 and 5.2), 2.88 (1H, br m, H-9a), 2.28 (1H, dq, H-3, *J* 12.0 and 7.4), 2.18–1.81, 1.72–1.50, 1.49–0.86 (5H, 4 × m, H-3a, H-4 and H-5), 1.87 (3H, s, C-9 CH_3), 1.26 (3H, d, C-3 CH_3, *J* 7.0) and 1.24 (3H, s, C-5a CH_3); δ_C (50 MHz; $CDCl_3$) 179.2 (C-2), 135.7 (C-8), 134.2 (C-9), 123.2 (C-6), 122.1 (C-7), 82.4 (C-9b), 46.5 (C-3), 46.1 (C-9a), 41.9 (C-5), 37.9 (C-5a), 32.5 (C-3a), 27.2 (C-4), 24.3 and 23.6 (C-9 CH_3 and C-5a CH_3) and 13.0 (C-3 CH_3); MS (EI, DIP) 232 (M^+, 54), 158 (41), 143 (69), 119 (38), 107 (37), 106 (100), 105 (52), 98 (55) and 91 (65).

Endoperoxide ***5b***

(3*S*,3a*S*,5a*S*,9a*R*,9b*S*)-3,5a,9-Trimethyl-3a,4,5,5a,9,9a-hexa hydro-3*H*-naphtho[1,2-*b*]furan-2-one **4** (279 mg, 1.20 mmol) was

dissolved in benzene (250 cm^3) in a quartz photochemical cell equipped with a high-pressure 150 W mercury-tungsten UV lamp in a quartz jacket. The jacket was cooled by circulating water. A steady stream of oxygen gas was delivered using a Pasteur pipette below the surface of the stirred solution. 5,10,15,20-Tetraphenyl-21*H*,23*H*-porphine (44.4 mg, 72.2 μmol) was added, affording a red solution. After thoroughly purging the solution with oxygen for about 15 min, the solution was irradiated for 18 h in the presence of oxygen, affording a green solution. Solids were filtered off, and the mixture concentrated to a green oil. Purification was achieved using radial chromatography [*c*-hexane – 1:2 (v/v) ethyl acetate : *c*-hexane gradient] gave α-*santonin* **3** (69.5 mg, 23%) followed by preparative thin-layer chromatography [using a 1:9–1:4 (v/v) ethyl acetate : *c*-hexane gradient], affording a brown oil, *endoperoxide* **5b** (88.8 mg, 28%); R_f 0.35 [1:4 (v/v) ethyl acetate : *c*-hexane]; $[\alpha_D]^{23}$ –40.1 (*c.* 1.55/MeOH). δ_H (400 MHz; $CDCl_3$) 6.69 (1H, dd, H-7, *J* 8.2 and 6.2), 6.34 (1H, dd, H-8, *J* 8.4 and 1.4), 4.13 (1H, dd, H-6, *J* 6.1 and 1.3), 4.08 (1H, dd, H-9b, *J* 11.6 and 6.6), 2.52 (1H, d, H-9a, *J* 6.6), 2.12 (1H, dq, H-3, *J* 11.6 and 7.0), 1.74 (1H, δ, $H\text{-}4_{ax}$, *J* 12.0, 6.6 and 5.4), 1.54–1.43 (2H, m, H-3a and $H\text{-}5_{eq}$), 1.47 (1H, t, $H\text{-}5_{ax}$, *J* 7.0), 1.40 (3H, s, C-9 CH_3), 1.37 (3H, s, C-5a CH_3), 1.37–1.26 (1H, m, $H\text{-}4_{eq}$) and 1.17 (3H, d, C-3 CH_3, *J* 6.8); δ_C (100 MHz; $CDCl_3$) 178.3 (C-2), 134.5 (C-8), 132.8 (C-7), 80.4 (C-9b), 80.1 (C-6), 76.98 (C-9), 49.3 (C-9a), 43.1 (C-3), 42.3 (C-5), 40.3 (C-5a), 30.9 (C-3a), 28.6 (C-5a CH_3), 23.5 (C-4), 21.1 (C-9 CH_3) and 12.5 (C-3 CH_3). Assignments were obtained and verified by C-H correlation, gCOSY, gHSQC and long-range ^2J-^3J coupling spectra.

*(3*S*,3a*S*,5a*S*,9*R**,9a*R*,9b*S*)-9-Methoxy-3-5a-9-trimethyloctahydro naphtho[1,2-*b*]furan-2,6-dione **13***

Endoperoxide **5b** (77.3 mg, 0.292 mmol) was dissolved in methyl alcohol (6 cm^3). To this solution was added sodium borohydride (46.1 mg, 1.22 mmol), with effervescence, and the mixture left to stir for 18 h at 50°C. The crude reaction mixture was then concentrated and purified by preparative thin-layer chromatography [using a 1:1 (v/v) ethyl acetate : *c*-hexane solution as eluent], affording an orange oil, *(3*S*,3a*S*,5a*S*,9*R**, 9a*R*,9b*S*)-9-methoxy-3-5a-9-trimethyloctahydronaphtho[1,2-*b*]furan-2,6-dione* **13** (41.0 mg, 53%); R_f 0.68 [1:1 (v/v) ethyl acetate : *c*-hexane]; $[\alpha_D]^{19}$ –13.7 (*c.* 0.80/MeOH). δ_H (200 MHz; $CDCl_3$) 3.94 (1H, br m, H-9b), 3.71 (3H, s, OCH_3), 2.73 (1H, dd, $H\text{-}7_{ax}$, *J* 19.1 and 3.3), 2.46 (1H, m, H-3), 2.48–2.35 (1H, m, $H\text{-}7_{eq}$), 2.23–2.05 (2H, m, $H\text{-}8_{ax}$ and H-9a), 1.86 (1H, d, $H\text{-}8_{eq}$, *J* 3.8), 1.79 (1H, br dt, $H\text{-}5_{eq}$, *J* 13.6 and 3.2), 1.62–1.38, 1.37–1.03 (4H, 2 × m, H-3a, H-4 and $H\text{-}5_{ax}$), 1.57 (3H, s, C-9 CH_3), 1.30 (3H, s, C-5a CH_3) and 1.17 (3H, d, C-3 CH_3, *J* 6.8); δ_C (50 MHz; $CDCl_3$) 217.2 (C-6), 176.9 (C-2), 79.0 (C-9b), 78.6 (OCH_3), 77.9 (C-9), 52.0 (C-5a), 49.0 (C-9a), 47.8 (C-7), 46.4 (C-3), 39.8 (C-3a), 39.3 (C-5), 33.1 (C-8), 30.2 (C-5a CH_3), 26.4 (C-4), 18.7 (C-9 CH_3) and 16.0 (C-3 CH_3); MS (EI, DIP) 296 (M^+, 8), 278 (10), 237 (23), 236 (35), 209 (75), 208 (93), 191 (36), 181 (31), 163 (29), 149 (100), 121 (68), 107 (33), 93 (50) and 88 (37).

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