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# Okundoperoxide, A Bicyclic Cyclofarnesylsesquiterpene Endoperoxide from *Scleria striatinux* with Antiplasmodial Activity

**Simon M. N. Efange**[*,†], **Reto Brun**[‡], **Sergio Wittlin**[‡], **Joseph D. Connolly**[§], **Thomas R. Hoye**[∞], **Thomas McAkam**[†], **Felix L. Makolo**[†], **James A. Mbah**[†], **Dorian P. Nelson**[∞], **Kennedy D. Nyongbela**[†], and **Clare K. Wirmum**[||]

[†]Department of Chemistry, University of Buea, P.O Box 63, Buea, Southwest Province, Cameroon [‡]Swiss Tropical Institute, Socinstrasse 57, CH-4002, Basel, Switzerland [§]Department of Chemistry, University of Glasgow, G12 8QQ, Scotland, UK [∞]Department of Chemistry, University of Minnesota, 207 Pleasant St., SE, Minneapolis, MN, 55455 USA [||]Medicinal Foods & Plants (MEFOPLA), Bamenda, Northwest Province, Cameroon

## Abstract

Okundoperoxide (**1**) was isolated by bioassay-guided fractionation of extracts from *Scleria striatinux* (syn. *S. striatonux*) (Cyperaceae). The compound contains a cyclic endoperoxide structural moiety and possesses moderate antimalarial activity.

In recent years, the clinical management of malaria has become more challenging due to progressive resistance to drugs such as chloroquine, which was once regarded as the mainstay of malaria chemotherapy.[1] The emerging resistance has fueled a search for new effective antimalarial agents. Exploration of natural products is a particularly attractive avenue of pursuit, in part because nature has provided the leads for the most successful drugs for the treatment of malaria during the past 60 years (quinine, quinidine, and/or their analogs), including, most recently, artemisinin. The identification of artemisinin as the main bioactive ingredient of *Artemisia annua* and the subsequent elaboration of the unique mechanism of action underlying its antiplasmodial activity[1b] have focused new attention on endoperoxides.[2] Endoperoxides are abundant in nature[2a] and display a broad range of pharmacological properties including antifungal, cytotoxic, antiviral, antitrypanosomal, and antiplasmodial activities.[1a] Herein, we report the isolation, structure elucidation, and antiplasmodial activity of (+)-(3*R**,4a*S**,8a*S**)-3-[(*E*)-4-hydroxybut-2-en-2-yl]-5,5,8a-trimethyl-3,4,4a,5-tetrahydrobenzo[c][1,2]dioxin-6(8a*H*)-one (okundoperoxide, **1**, Figure 1), a new and skeletally unique bicyclofarnesyl sesquiterpene endoperoxide. This secondary metabolite was obtained from the roots of *Scleria striatinux* de Wild (syn. *S. striatonux*) [Cyperaceae], a local spice in parts of Cameroon. The roots of *S. striatinux* are also used as an herbal tea for fevers. This study was prompted by the observation that a $CH_2Cl_2$/MeOH extract of *S. striatinux* was moderately active against both chloroquine-sensitive and -resistant strains of *Plasmodium falciparum* (cf., Table 2, entry 1).

A sample of *S. striatinux* roots was harvested in Oku in the Northwest Province of Cameroon. Following gradient chromatography on silica gel and subsequent size exclusion chromatography (SEC) on Sephadex LH-20, the extract of the dried roots gave okundoperoxide (**1**, $R_f$ 0.3 in 3:2 hexanes:EtOAc). Okundoperoxide was unstable to gas chromatographic

[*]To whom correspondence should be addressed: smnefange@yahoo.com.

analysis,[3] an observation that became understandable once the peroxide subunit was identified. High resolution ESI-MS analysis gave a sodiated parent (and base peak) ion of mass 289.1402. The $^{13}C$ NMR spectrum in $CDCl_3$ indicated the presence of 15 carbon atoms. Together, these data showed a molecular formula of $C_{15}H_{22}O_4$ and five degrees of unsaturation.

Key $^1H$ and $^{13}C$ NMR data are reported in Table 1. All 15 carbon and 22 (first order) proton resonances were identified. The $^{13}C$ NMR spectrum contained resonances for one ketone and four olefinic carbons. The $^1H$ NMR spectrum suggested the presence of four methyl groups (one allylic with only long-range coupling and three aliphatic singlets) and three olefinic, one oxymethine, and one pair of oxymethylene protons. The HMQC spectrum clearly showed one-bond correlations that are the primary basis for the assignments of carbon chemical shifts listed in Table 1.

The IR spectrum showed characteristic absorption bands for hydroxy (3477 $cm^{-1}$) and carbonyl (1674 $cm^{-1}$) groups. The former was consistent with a one-proton resonance at δ 1.36 ppm, which disappeared in a deuterium exchange experiment. The carbonyl absorption was suggestive of a conjugated enone, which was supported in the NMR spectrum by the chemical shifts of olefinic proton (δ 6.73 and 5.94) and carbon (δ 150.3 and 127.9) signals and of the carbonyl carbon resonance (δ 203.2). These data, together with the doublets of the olefinic proton resonances ($J$ = 10.2 Hz), pointed to a 4,4-disubstituted *Z*-enone moiety.

The $^1H$-$^1H$ COSY spectrum indicated an isolated four spin system that included the proton at δ 1.96, having three large coupling constants (13.0, 13.0, 11.2 Hz). This was indicative of an axial-like methylene proton in a six-membered ring, flanked by two vicinal, *trans* methine protons (-CHC$\underline{H}_{ax}H_{eq}$CH-). A COSY correlation between resonances for the olefinic proton at δ 5.75 and the methylene pair centered at δ 4.26 indicated a trisubstituted olefin bearing an oxymethylene group. The connectivity pattern deduced from the HMBC spectrum integrated the above subunits, along with the four methyl groups, into a common constitution. Specifically, structure **1** was consistent with all of the COSY and HMBC correlation data.

In addition to the 1,3-diaxial nature of H-4 and H-6 deduced from the coupling constant analysis, the remaining relative configurations shown in **1** were assigned largely on the basis of NOE observations (Figure 2). The acyclic *E*-olefin geometry is indicated by the enhancement of H-1 by H-15.[4] Mutual enhancements of H-4 and H-6 reaffirm their cis-relationship. The *trans* nature of the ring fusion was deduced from the sets of NOEs among H-$5_{ax}$/H-12/H-14 and H-4/H-$5_{eq}$/H-6/H-13.

With the intent of reducing the peroxide bond in **1** with triphenylphosphine via an intermediate like **6** (Scheme 1), we treated a sample of **1** with $Ph_3P$ in $CDCl_3$ and monitored the subsequent events by $^1H$ NMR spectroscopy. Somewhat surprisingly, there was no observable change at ambient temperature. Moreover, when the reaction solution was heated in a 65 °C bath, the major product formed was the furan **2**, which has the same overall oxidation state as **1** and is the result of a net dehydration reaction. We suspect that enone **3** is an intermediate in this transformation. Zwitterion **6**, if formed, could preferentially undergo intramolecular elimination of phosphine (see arrows in **6**) rather than, for example, cyclization to a fused tetrahydrofuran derivative via displacement of triphenylphosphine oxide. Alternatively, the hindered nature of the dialkylperoxide in **1** may have induced a different reaction course from the outset. Namely, the phosphine may have functioned preferentially as a base rather than as a reductant to effect an eliminative opening via loss of H-4 and cleavage of the peroxide O-O bond (see arrows in **1**) to give the enone **3**.[5] *E*-γ-Hydroxy-α,β-enones similar to **3** are known to undergo spontaneous isomerization and dehydration reactions to give furans.[5,6] Enone *E*- to *Z*-isomerization to convert **3** to **4** could involve a reversibly formed, rotatable intermediate epoxide (*cf.* **7a**) or triphenylphosphine adduct (*cf.* **7b**). There are many reported examples of

dehydration of *Z*-γ-hydroxy-α,β-enones like **4** under mild conditions to give the corresponding furans,[7] likely via hemiketals like **5**. It is notable that among the many thermal decomposition products observed upon GC-MS analysis of okundoperoxide (**1**), the furan **2** was the most abundant.[3]

Finally, the *R*- and the *S*-Mosher ester (methoxytrifluoromethylphenylacetyl, MTPA) derivatives of the alcohol **1** (**8R** and **8S**, respectively, in Figure 3) were prepared using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and the *R*- and *S*-Mosher acid (MTPA-OH), respectively. The $^1$H NMR data for these esters do not allow us to deduce the absolute configuration of **1** because of the large distance between the MTPA and substrate stereogenic centers. However, the spectra of these diastereomers are distinguishable, which should be helpful for later assignment of absolute configuration upon synthesis of one enantiomer of **1**, an endeavor we are pursuing.

Antiplasmodial activity for the initial crude extract and for purified okundoperoxide (**1**) are shown in Table 2. Although the sample of **1** used for the biological testing was only 90% pure, we attribute the observed antiplasmodial activity to **1** because the interfering material, which was also isolated from a partially overlapped chromatographic fraction, is inactive in this assay. Finally, the presence of other compounds with antiplasmodial activity in *S. striatinux* cannot be ruled out. Further investigation of this possibility is also ongoing.

## Experimental Section

### General Experimental Procedures

Analytical TLC was performed using silica gel plates containing F254 indicator, and the plates were visualized by staining with ceric ammonium nitrate or iodine. Low pressure open-column chromatography was carried out with glass columns using silica gel (60-200 mesh, JT Baker) as stationary phase. Size exclusion chromatography was performed with Sephadex LH-20 (Sigma Aldrich). Purification by medium pressure liquid chromatography (MPLC) was done on columns that were hand packed with 25-32 μm silica gel and monitored with a refractive index detector. Final purification was performed on an Econosil HPLC silica gel column (10 μm, 10 mm × 250 mm) equipped with a refractive index detector.

$^1$H NMR spectra (1D, COSY, HMQC and HMBC) were acquired on a Varian V1-500 (500 MHz) spectrometer. 13C NMR spectra were (1D) were acquired on a Varian V1-300 (75.4 MHz) spectrometer. Samples were prepared in $CDCl_3$ containing tetramethylsilane (TMS). Chemical shifts (δ) are reported in ppm and referenced to TMS (δ = 0 ppm) for $^1$H and (δ = 77 ppm) for $^{13}$C NMR spectra. Coupling constants (*J*) are reported in Hz. Infrared spectra were recorded on a Prospect 4000 FT-IR spectrophotometer (Midac Corporation), and only the most intense and/or diagnostic peaks are reported. All samples were thin films on a NaCl window. HRMS were recorded on a Bruker Biotof II (ESI-TOF) instrument using PEG or PPG as an internal calibrant. Low-resolution GC-MS data were collected on an Agilent 5975 XL-MSD instrument at 70 eV fitted with an HP-5 column (15 m in length).

### Compound Isolation

The sample of *S. striatinux* roots was harvested in Oku in the Northwest province of Cameroon. Plant identification was carried out in collaboration with botanists at the Limbe Botanical and Zoological Gardens and the Cameroon National Herbarium, Yaounde, Cameroon. A voucher sample (No. 32235/HNC) has been deposited at the Cameroon National Herbarium.

The roots were air-dried, ground to a powder (10 kg), and macerated in $CH_2Cl_2$/MeOH (1:1) for 6 days. After removal of the extract by decantation and subsequent filtration, fresh solvent was added to the marc and the process was repeated once. The combined extracts were

concentrated under reduced pressure to provide the crude extract (450 g). The material was chromatographed on a silica gel column and eluted with a stepwise gradient of EtOAc/hexanes (0, 5, 10, 20, 40, 60, 80%) and then with MeOH to yield eight fractions. The fraction that eluted with EtOAc/hexanes (40:60) was rechromatographed twice on Sephadex LH-20, using, first, $CH_2Cl_2$ and then hexanes/$CH_2Cl_2$ (7:3) elution to yield 1 g of **1** (0.01 % yield) as an oil having a purity of ca. 90% (by $^1$H NMR assay). Biological assays were carried out with this sample. [The closely migrating material, which constitutes *ca*. 10% of the sample, was subsequently purified, tested, and found to be inactive in the antiplasmodial assay.] Further purification of a portion of this sample by normal phase HPLC (2:1 hexanes:EtOAc, 10 μm silica, 10 × 250 mm) gave the material on which the spectroscopic data reported here for **1** were collected. IR (thin film): 3477 (OH), 2989 ($C_{sp3}$H), 2937 ($C_{sp3}$H), 2879 ($C_{sp3}$H), and 1674 (C=O) cm$^{-1}$; NMR (see Table 1); HRMS (ESI, [*M*+Na]$^+$, *m/z*): found 289.1402, calcd. 289.1410; and $[\alpha]^{21}_D$ = 72.9 ($CHCl_3$, c = 1.6 g/100 mL).

### Preparation of 2 from okundoperoxide (1)

The alcohol **1** (8.0 mg) was dissolved in $CDCl_3$ (0.7 mL) in an NMR tube, and $Ph_3P$ (8.7 mg, 1.1 equiv) was added to the solution. The tube was capped and heated in an oil bath at 65 °C for 20 hours. The solution was concentrated and purified by MPLC (2:1 hexanes:EtOAc) to give 1.5 mg of furan **2**. $^1$H NMR (500 MHz, $CDCl_3$): δ 1.13 (s, Me-12), 1.15 (s, Me-13), 1.46 (s, Me-14), 2.02 (s, Me-15), 2.52 (dd, *J* = 7.9, 5.5 Hz, H-6), 2.82 (dd, *J* = 15.5, 5.5 Hz, H-5a), 2.95 (dd, *J* = 15.5, 7.9 Hz, H-5b), 5.88 (d, *J* = 10.2 Hz, H-9), 6.18 (d, *J* = 1.9 Hz, H-2), 6.69 (d, *J* = 10.2 Hz, H-8), and 7.25 (d, *J* = 1.9 Hz, H-1); $^{13}$C NMR (75 MHz, $CDCl_3$): δ = 10.0 (C-15), 21.0 (C-12), 22.0 (C-5), 23.6 (C-14), 24.7 (C-13), 45.2 (C-11), 52.7 (C-6), 71.4 (C-7), 113.4 (C-2), 113.4 (C-3), 125.2 (C-9), 140.1 (C-1), 149.4 (C-4), 154.6 (C-8), and 203 (C-10; chemical shift taken from the HMBC spectrum); IR (thin film): 3439 (OH), 2976 ($C_{sp3}$H), 2926 ($C_{sp3}$H), 2855 ($C_{sp3}$H), and 1674 (C=O) cm$^{-1}$; GC-MS *m/z* (%): 248 (8), 230 (9), 215 (9), 202 (6), 187 (7), 160 (37), 135 (10), 121 (6), 107 (9), 98 (31), 97 (17), 96 (15), 95 (100), 91 (11), 79 (7), 77 (7), 70 (6), 69 (7), 67 (8), 65 (8), 55 (15), and 53 (6); HRMS [*M*+Na]$^+$, *m/z*: found 271.1311, calcd. 271.1305.

### Preparation of the (*R*)-Mosher ester 8R

The alcohol **1** (5.0 mg) was taken up in $CH_2Cl_2$ (0.2 mL) and DMAP (6.9 mg, 3.0 equiv), EDC (5.4 mg, 1.5 equiv), and (*R*)-(+)-MTPA-OH [(*R*)-Mosher acid, 6.6 mg, 1.5 equiv] were added sequentially. After being stirred at rt for 3 h, the solution was concentrated. The residue was passed through a silica plug (ca. 6 mm d × 5 cm h; 3:1 hexanes:EtOAc) to give ~3 mg of the (*R*)-Mosher ester **8R**: $^1$H NMR (500 MHz, $CDCl_3$, TMS reference): δ = 1.08 (s, Me-12), 1.19 (s, Me-13), 1.58 (s, Me-14), 1.66 (dddd, *J* = 13.2, 2.7, 2.7, 0.5 Hz, H-5b), 1.80 (s, Me-15), 1.88 (ddd, *J* = 13.0, 13.0, 11.2 Hz, H-5a), 2.44 (dd, *J* = 12.9, 3.3 Hz, H-6), 3.55 (q, *J* = 1.2 Hz, $OCH_3$), 4.57 (br dd, *J* = 11, 2 Hz, H-4), 4.88 (br d, *J* = 6.8 Hz, $CH_2$-1), 5.68 (tdq, *J* = 6.9, 1.2, 1.2 Hz, H-2), 5.95 (d, *J* = 10.2 Hz, H-9), 6.73 (dd, *J* = 10.2, 0.6 Hz, H-8), 7.38-7.43 (m, 3H, ArH), and 7.50-7.53 (m, 2H, ArH).

### Preparation of the (*S*)-Mosher ester 8S

The (*S*)-Mosher ester was independently prepared in an analogous fashion as the *R*-ester, but using (*S*)-(-)-MTPA-OH [(*S*)-Mosher acid] instead. (*S*)-Mosher ester **8S**: $^1$H NMR (500 MHz, $CDCl_3$, TMS reference): δ = 1.08 (s, Me-12), 1.19 (s, Me-13), 1.58 (s, Me-14), 1.66 (dddd, *J* = 13.1, 2.7, 2.7, 0.7 Hz, H-5b), 1.80 (s, Me-15), 1.88 (ddd, *J* = 13.0, 13.0, 11.2 Hz, H-5a), 2.44 (dd, *J* = 12.8, 3.3 Hz, H-6), 3.56 (q, *J* = 1.2 Hz, $OCH_3$), 4.57 (br dd, *J* = 11.3, 2.4 Hz, H-4), 4.87 (dd, *J* = 12.7, 6.9 Hz, C$\underline{H}_a$H$_b$-1), 4.89 (dd, *J* = 12.7, 6.9 Hz, CH$_a\underline{H}_b$-1), 5.67 (tdq, *J* = 6.8, 1.3, 1.3 Hz, H-2), 5.95 (d, *J* = 10.2 Hz, H-9), 6.72 (dd, *J* = 10.2, 0.7 Hz, H-8), 7.38-7.43 (m, 3H, ArH), and 7.50-7.53 (m, 2H, ArH).

### Biological testing

The *S. striatinux* crude extract was tested at the Walter Reed Army Institute of Research (WRAIR), Washington, DC. Compound **1** was tested at both WRAIR and at the Swiss Tropical Institute (STI), Basel, Switzerland. The samples were tested following the [$^3$H]hypoxanthine incorporation assay described by Desjardins *et al*.[10]

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

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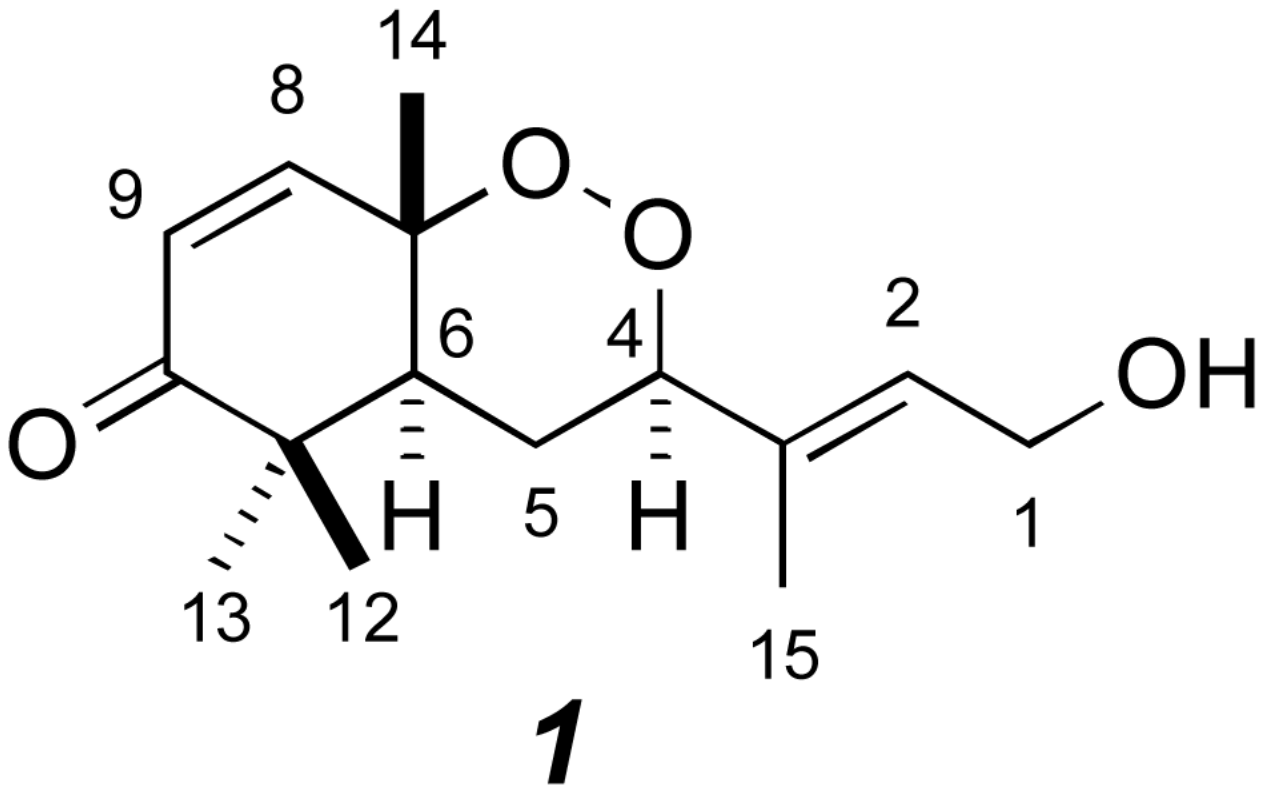



**Figure 1.**
Structure and relative configuration of okundoperoxide (**1**). The absolute configuration of **1** is undetermined; the structure of the enantiomer portrayed has been arbitrarily chosen.

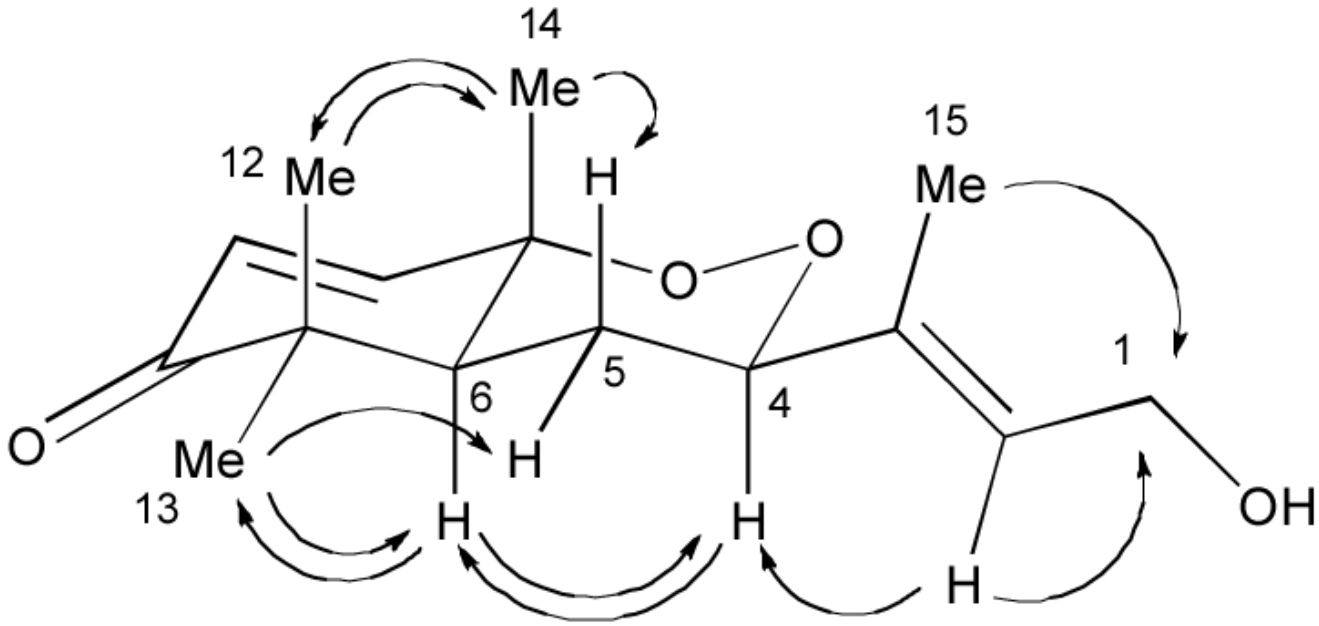



**Figure 2.**
Three-dimensional representation of **1** with key NOE correlations shown; these were used to assign the relative configurations among the three stereogenic $C_{sp3}$-centers and the geometry of the acyclic alkene.

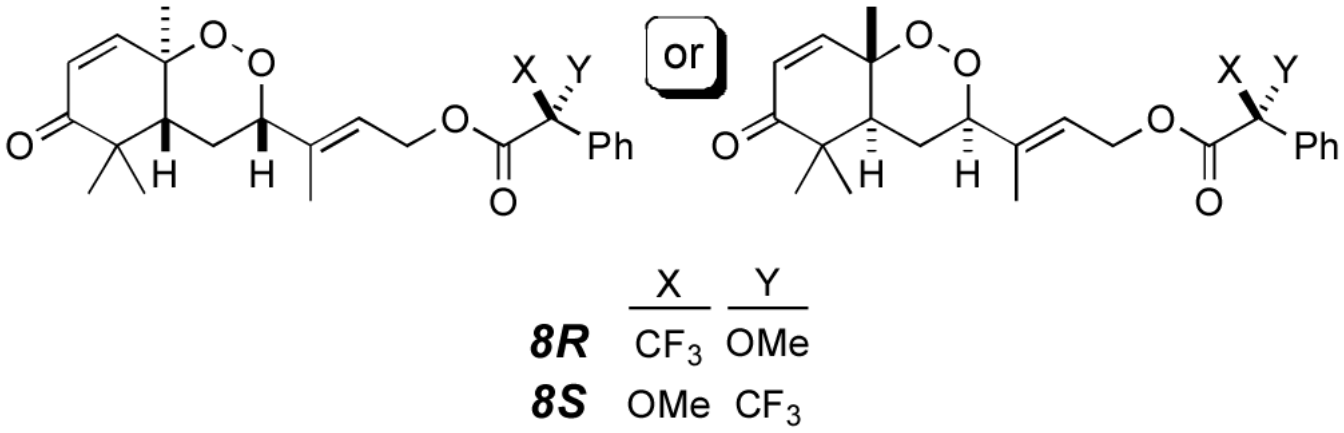
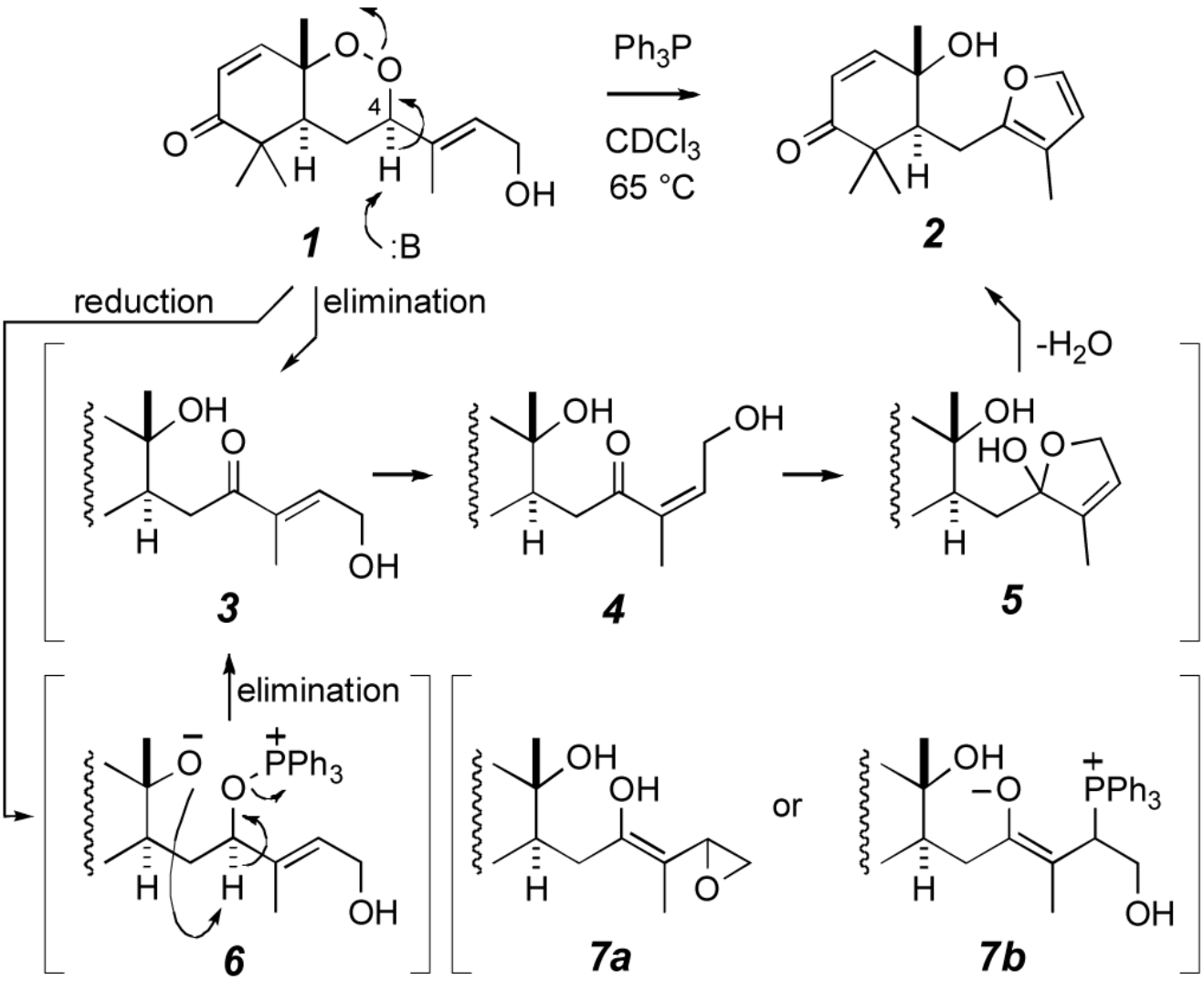



**Figure 3.**
The two possible structures for each of **8R** and **8S**, the *R*- and *S*-Mosher ester derivatives of **1**, prepared[8] via EDC coupling of **1** with *R*- and *S*-MTPA-OH, respectively.




**Scheme 1.**

**Table 1**

$^{13}C$ and $^{1}H$ NMR Spectroscopic Data for Okundoperoxide (**1**) ($CDCl_3$, 75 and 500 MHz)

| Atom number | Carbon | Proton | | | COSY | HMBC |
|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | mult | *J* [Hz] | (to $^{1}H$-#) | (from $^{1}H \rightarrow {}^{13}C$-#) |
| 1 | 59.0 | 4.26 | br dd | 5.5, 5.5 | H-2, H-15 | C-2, C-3 |
| 2 | 128.3 | 5.75 | tdq | 6.4, 1.3, 1.3 | H-1, H-15 | C-1, C-4, C-15 |
| 3 | 135.0 | | | | | |
| 4 | 86.6 | 4.56 | br dd | 11.2, 2.7 | H-5a, H-5b | C-2, C-3, C-15 |
| 5ax | 24.7 | 1.96 | ddd | 13.0, 13.0, 11.2 | H-4, H-5b, H -6 | C-3, C-4, C-6, C-7 |
| 5eq | | 1.70 | dddd | 13.2, 3.0, 2.5, 0.8 | H-4, H-5a, H-6 | C-6, C-7 |
| 6 | 49.3 | 2.45 | dd | 12.9, 3.3 | H-5a, H-5b | C-5, C-7, C-11, C-12, C-13 |
| 7 | 79.4 | | | | | |
| 8 | 150.3 | 6.73 | dd | 10.2, 0.8 | H-9 | C-6, C-10 |
| 9 | 127.9 | 5.94 | d | 10.2 | H-8 | C-7, C-11 |
| 10 | 203.2 | | | | | |
| 11 | 43.5 | | | | | |
| 12 | 20.5 | 1.09 | s | | | C-6, C-10, C-11, C-13 |
| 13 | 26.0 | 1.19 | s | | | C-6, C-10, C-11, C-12 |
| 14 | 21.1 | 1.59 | s | | | C-6, C-7, C-8 |
| 15 | 13.8 | 1.76 | dt | 1, 1 | H-1, H-2 | C-2, C-3, C-4 |
| OH | | 1.36 | br t | 5.3 | | |

**Table 2**

Antiplasmodial Activity of *S. striatinux:* Crude Extract Versus Secondary Metabolite

| Sample | $IC_{50}$ (ng/mL) | | | | |
|---|---|---|---|---|---|
| | W2[a] | D6[a] | K1[b] | NF54[b] | Cytotoxicity |
| *S. striatinux* (crude extract) | 804 | 894 | NT[c] | NT[c] | NT[c] |
| Okundoperoxide (**1**) | 470 | 483 | 1498 | 1308 | 22,700 |
| Chloroquine (control) | 84 | 3 | 62[d] | 5.1[d] | NT[c] |
| Podophyllotoxin | - | - | - | - | 7 |

[a] Data obtained at the Walter Reed Army Institute of Research; W2 is a chloroquine-resistant and D6 a chloroquine-sensitive strain of *Plasmodium falciparum*.

[b] Data obtained at the Swiss Tropical Institute. K1 is a chloroquine- and pyrimethamine-resistant strain of *Plasmodium falciparum* from Thailand. NF54 is a drug sensitive airport strain of unknown origin. Data presented as the mean of 2 or 3 determinations. Individual measurements generally differed by less than 50%.

[c] NT, not tested.

[d] From ref 9.