MDN-0104, an Antiplasmodial Betaine Lipid from Heterospora chenopodii

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · SEPTEMBER 2014

Impact Factor: 3.8 · DOI: 10.1021/np500577v · Source: PubMed

JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

MDN-0104, an Antiplasmodial Betaine Lipid from *Heterospora chenopodii*

Jesús Martín,[†] Gloria Crespo,[†] Víctor González-Menéndez,[†] Guiomar Pérez-Moreno,[‡] Paula Sánchez-Carrasco,[‡] Ignacio Pérez-Victoria,*[,†] Luis M. Ruiz-Pérez,[‡] Dolores González-Packanowska,[‡] Francisca Vicente,[†] Olga Genilloud,[†] Gerald F. Bills,[†,§] and Fernando Reyes*[,†]

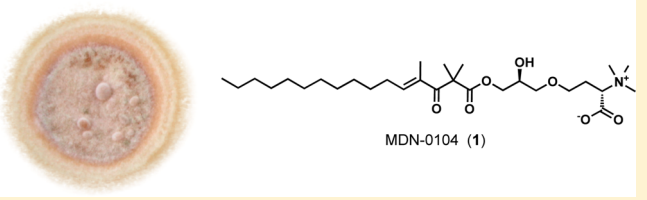
[†]Fundación MEDINA, Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía, Avenida del Conocimiento 34, Parque Tecnológico de Ciencias de la Salud, E-18016, Granada, Spain

[‡]Instituto de Parasitología y Biomedicina López-Neyra, CSIC, Avenida del Conocimiento s/n, Parque Tecnológico de Ciencias de la Salud, E-18100 Armilla, Granada, Spain

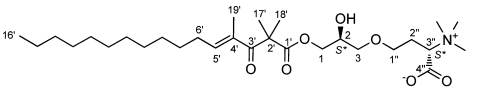
[§]Texas Therapeutics Institute, The Brown Foundation Institute of Molecular Medicine, The University of Texas Health Science Centre at Houston, Houston, Texas 77054, United States

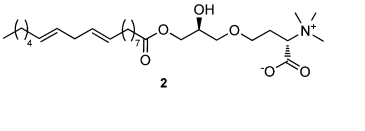
Supporting Information

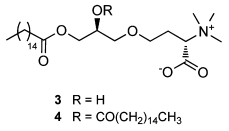
ABSTRACT: Bioassay-guided fractionation of the crude fermentation extract of *Heterospora chenopodii* led to the isolation of a novel monoacylglyceryltrimethylhomoserine (**1**). The structure of this new betaine lipid was elucidated by detailed spectroscopic analysis using one- and two-dimensional NMR experiments and high-resolution mass spectrometry. Compound **1** displayed moderate *in vitro* antimalarial activity against *Plasmodium falciparum*, with an IC_{50} value of 7 μM. This betaine lipid is the first monoacylglyceryltrimethylhomoserine ever reported in the Fungi, and its acyl moiety also represents a novel natural 3-keto fatty acid. The new compound was isolated during a drug discovery program aimed at the identification of new antimalarial leads from a natural product library of microbial extracts. Interestingly, the related fungus *Heterospora dimorphospora* was also found to produce compound **1**, suggesting that species of this genus may be a promising source of monoacylglyceryltrimethylhomoserines.

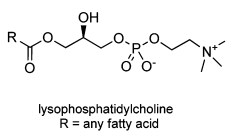


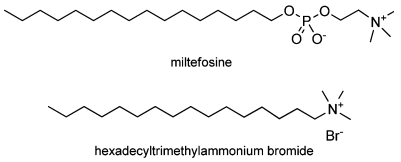
Ether-linked glycerolipids containing a betaine moiety occur naturally in algae, bryophytes, fungi, and some primitive protozoa and photosynthetic bacteria.[1−7] They are not found in flowering plants, but have been detected in some spore-producing plants.[3] These complex lipids contain a polar group linked by an ether bond at the *sn*-3 position of the glycerol moiety, with fatty acids esterifying the *sn*-1 and *sn*-2 positions. Three related classes of these lipids differing in the permethylated hydroxyamino acids linked to diacylglycerols through an ether bond have been described. They all possess a positively charged trimethylammonium group and a negatively charged carboxyl group and therefore are zwitterionic at neutral pH. The three types of betaine lipids include 1,2-diacylglyceryl-3-*O*-4′-(*N*,*N*,*N*-trimethyl)homoserine (DGTS), 1,2-diacylglyceryl-3-*O*-2′-(hydroxymethyl)(*N*,*N*,*N*-trimethyl)-β-alanine (DGTA), and 1,2-diacylglyceryl-3-*O*-carboxy-(hydroxymethyl)-choline (DGCC).[7] Of these, the diacylglyceryltrimethylhomoserines are by far the most common in nature, and taxonomic studies suggest that they may have been the evolutionary progenitor of betaine lipids.[3] On the other hand, only two monoacylglyceryltrimethylhomoserines have been reported to date (**2**, **3**) and were isolated as an inseparable mixture from the green alga *Ulva fasciata* collected from the Indian coastline. Betaine lipid **3** was the major component of the mixture in which the related DGTS **4** was also present.[8]











Malaria is an infectious disease caused by parasites belonging to the genus *Plasmodium*. Two billion people live in areas at risk

Received: July 21, 2014

dx.doi.org/10.1021/np500577v | *J. Nat. Prod.* XXXX, XXX, XXX−XXX

from the disease, and annually up to one million people die from malaria infection.[9,10] The discovery and development of new drugs for the treatment of malaria is thus of major importance. Historically, medicinal plants have played a very important role in combating this disease. The malaria-active metabolites quinine and artemisinin represent the best-known examples of natural products that have led to the development of numerous antimalarial drugs.[11] However, the prevalence of resistance of the malaria parasite to known drugs and the lack of efficacy of some of these have highlighted the urgent need for the discovery and development of new antimalarials developed from unique chemical structures based on different modes of action.[12,13] Microbial secondary metabolites cover a broad chemical space[14,15] and offer an excellent opportunity for finding possible antiplasmodium leads.[16] Fundación MEDINA has initiated a drug discovery program aimed at the identification of new antimalarial leads from its proprietary library of microbial (fungi, actinomycetes, and other bacteria) natural product extracts. In this article, we report the isolation and structure elucidation of MDN-0104, a novel monoacylglyceryltrimethylhomoserine (**1**) containing as the acyl moiety a new unusual 3-keto fatty acid. Its *in vitro* antimalarial activity against *Plasmodium falciparum* is also reported herein. This new betaine lipid has been isolated from a fermentation broth of the fungus *Heterospora chenopodii* (Ascomycota, Pleosporales, Leptosphaeriaceae).

RESULTS AND DISCUSSION

In one of our screening campaigns, *in vitro* antiplasmodial activity against *P. falciparum Pf* 3D7 was detected in the acetone extract corresponding to the microfermentation of the fungus *H. chenopodii* in 1 mL of STP medium, one of eight media included in our nutritional array microfermentations.[17−21] The strain employed (CBS 109836) was originally isolated from *Atriplex prostrata* collected at Wissenkerke, Zeedijk Oosterschelde, near Westnol (Netherlands) and deposited at the Fungal Biodiversity Centre by G. Verkley. Interestingly, *H. chenopodii* was first described as *Phyllosticta chenopodii*[22] and recently reclassified in the genus *Heterospora* after molecular and phylogenetic studies, which led to the conclusion that *Phoma* should be restricted to *Didymellaceae*.[23] This revision also led to the redisposition of another *Phoma*-like anamorph associated with plants of the Chenopodiaceae, *P. heteromorphospora*, a sister species of *H. chenopodii*, which was transferred to the genus *Heterospora* as *H. heteromorphospora*.[23] LC-UV-MS analysis of the corresponding extract did not identify any known bioactive microbial metabolite included in our in-house library.[21,24−26] A bioassay-guided chromatographic fractionation strategy was then pursued to isolate the bioactive compound. The culture was scaled up to 600 mL in a static fermentation of the same medium. Fractionation of the acetone extract of the broth on SP-207ss resin followed by semipreparative HPLC led to the isolation of a new monoacylglyceryltrimethylhomoserine, MDN-0104 (**1**), as the compound responsible for the biological activity.

Compound **1** was assigned a molecular formula of $C_{29}H_{53}NO_7$ using HRESIMS and MS/MS. No hit was retrieved when searching this formula in the Dictionary of Natural Products database,[27] suggesting the novelty of the compound. Analysis of the ^{1}H NMR and ^{13}C NMR spectra (Table 1) alongside the HSQC spectrum allowed the identification of signals corresponding to two olefinic carbons (one quaternary plus a methine), two aliphatic methines, 14 methylene groups

Table 1. NMR Spectroscopic Data (500 MHz, CD_3OD, at 24 °C) for MDN-0104 (1)

position	δ_C, type	δ_H (J in Hz)	COSY	HMBC (^{1}H to ^{13}C)
1	67.2, CH_2	a 4.14, dd (11.1, 5.5)	1b, 2	1′, 2, 3
		b 4.10, dd (11.1, 4.9)	1a, 2	1′, 2, 3
2	69.3, CH	3.89, quintet (5.3)	1a, 1b, 3a, 3b	1, 3
3	73.4, CH_2	a 3.46, dd (10.0, 4.5)	3b, 2	1, 2, 1″
		b 3.41, dd (10.0, 5.9)	3a, 2	1, 2, 1″
1′	176.6, C			
2′	54.0, C			
3′	200.7, C			
4′	135.7, C			
5′	144.2, CH	6.43, td (7.2, 0.9)	6′, 19′	3′, 6′, 7′, 19′
6′	29.8, CH_2	2.24, m	5′, 7′, 19′	4′, 5′, 7′, 8′
7′	29.6, CH_2	1.45, t (6.8)	6′, 8′	5′, 6′, 8′, 9′
8′	30.4, CH_2	1.33, m	7′, 9′	6′, 7′, 9′, 10′
9′	30.5,[a] CH_2	1.41, m	8′, 10′	7′, 8′, 10′, 11′
10′	30.7,[a] CH_2	1.41, m	9′, 11′	8′, 9′, 11′, 12′
11′	30.7,[a] CH_2	1.41, m	10′, 12′	9′, 10′, 12′, 13′
12′	30.7,[a] CH_2	1.41, m	11′, 13′	10′, 11′, 13′, 14′
13′	30.5,[a] CH_2	1.41, m	12′, 14′	11′, 12′, 14′, 15′
14′	33.0, CH_2	1.29, m	13′, 15′	12′, 13′, 15′, 16′
15′	23.7, CH_2	1.32, m	14′, 16′	13′, 14′, 16′
16′	14.4, CH_3	0.90, t (6.9)	15′	14′, 15′
17′	25.1, CH_3	1.41, s		1′, 2′, 3′, 18′
18′	25.1, CH_3	1.41, s		1′, 2′, 3′, 17′
19′	12.6, CH_3	1.76, br s	5′, 6′	3′, 4′, 5′, 6′
1″	68.6, CH_2	a 3.64, dt (9.5, 4.7)	1a″, 2a″, 2b″	3, 2″, 3″
		b 3.55, td (9.5, 4.3)	1b″, 2a″, 2b″	3, 2″, 3″
2″	29.0, CH_2	a 2.23, m	2b″, 1a″, 1b″, 3″	1″, 3″, 4″
		b 2.08, m	2a″, 1a″, 1b″, 3″	1″, 3″, 4″
3″	77.7, CH	3.75, dd (11.3)	2a″, 2b″	1″, 2″, 4″, N-CH_3
4″	171.8, C			
N-CH_3	52.4, CH_3	3.22, s		3″

[a] ^{13}C assignments may be interchanged.

including three oxygenated methylenes, seven methyls, and three carbonyl signals (δ_C 200.7, 176.6, and 171.8 ppm). A strong singlet signal corresponding to three equivalent methyl groups at δ_H 3.22 ppm in ^{1}H NMR spectrum, with correlation in HSQC at δ_C 52.4 ppm, suggested the presence of a trimethylammonium group. In-depth analysis of the 2D NMR data including additional COSY and HMBC spectra led to the identification of a trimethylhomoserine unit with ^{13}C NMR signals at δ_C 68.6 (C-1″), 29.0 (C-2″), 77.7 (C-3″), 171.8 (C-4″), and 52.4 (N-CH_3) ppm. Similarly, a glycerol moiety was also revealed with ^{13}C NMR signals at δ_C 67.2 (C-1), 69.3 (C-2), and 73.4 (C-3) ppm. The HMBC correlation (Figure 1) between the methylene protons of the homoserine moiety at position C-1″ with the methylene carbon of glycerol at δ_C 73.4 ppm (C-3) and *vice versa* suggested that the homoserine unit was attached to the glycerol moiety at C-3 via an ether bond. The carbonyl carbon at δ_C 176.6 ppm (C-1′) displayed an HMBC correlation with the glycerol methylene at position C-1,

dx.doi.org/10.1021/np500577v | J. Nat. Prod. XXXX, XXX, XXX−XXX

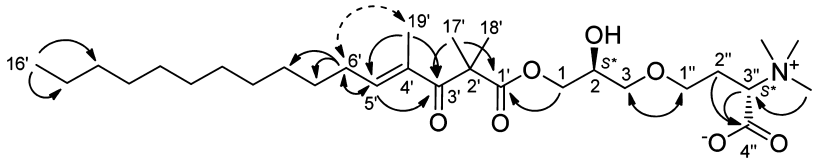
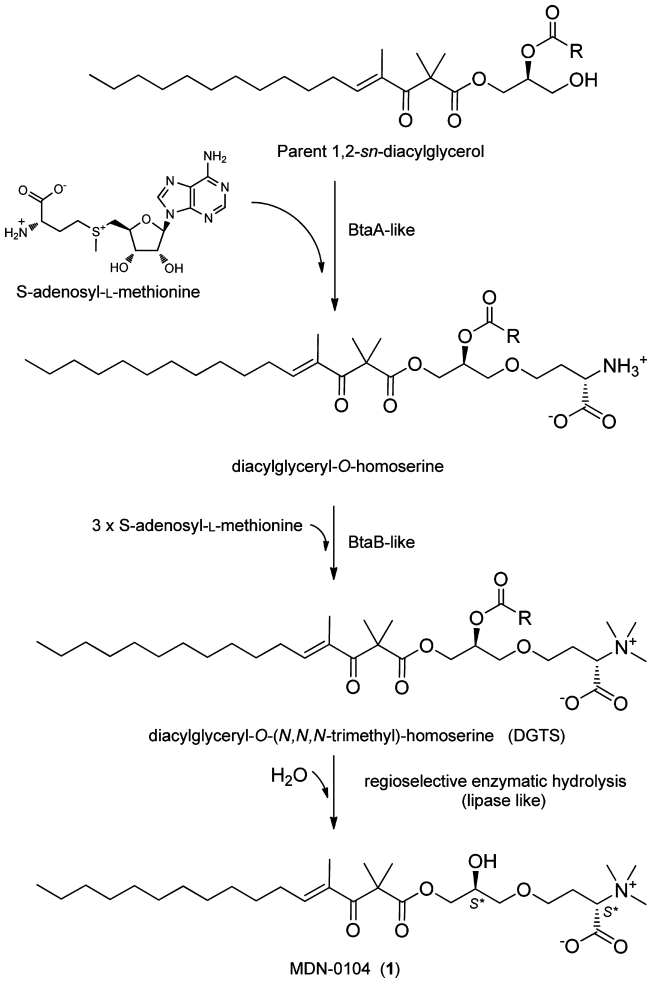


Figure 1. Key HMBC (solid arrows) and NOESY (dashed arrow) correlations observed for **1**.

suggesting the acylation of this methylene, which was also confirmed by the low-field chemical shift of both H-1 diastereotopic protons (δ_H 4.14 and 4.10 ppm). On the other hand the glyceryl methine proton (H-2) only had HMBC correlations with the other two glyceryl methylenes (C-1 and C-3). Thus, compound **1** was identified as a monoacylglyceryltrimethylhomoserine. The connectivity of the fatty acid moiety was established starting with the key HMBC correlations (Figure 1) observed for the carbonyl carbon of the ester group (δ_C 176.6 ppm). Apart from the already mentioned correlation with the corresponding glyceryl methylene, this carbonyl displayed an additional correlation with the singlet signal corresponding to two isochronous methyl groups at δ_H 1.41 ppm. The HMBC correlation observed for these methyl groups at their own frequency (δ_C 25.1 ppm) confirmed their geminal location at position α with respect to the ester carbonyl. Those methyl groups also gave HMBC correlations with the quaternary carbon they are directly bonded to (δ_C 54.0 ppm) and with a ketone carbonyl at δ_C 200.7 ppm, indicating the 3-oxo (i.e., β-keto) nature of the fatty acid. The ketone carbonyl also showed HMBC correlations with a broad methyl singlet at δ_H 1.76 ppm and with the only olefinic proton found at δ_H 6.43 ppm (triple doublet). The methyl singlet displayed additional HMBC correlations with two olefinic carbons (the methine at δ_C 144.2 ppm and the quaternary carbon at δ_C 135.7 ppm) but no correlation with any aliphatic methylene, confirming its location at position α with respect to the keto functionality and indicating that this carbonyl was α,β-unsaturated. The COSY spectrum clearly displayed two long-range correlations for this methyl group, one due to allylic coupling with the olefinic proton and the second due to homoallylic coupling with the methylene vicinal to the unsaturation. Analysis of the 2D NMR spectra revealed that the methylene directly bonded to the unsaturation (C-6′) was linked to a nonbranched aliphatic chain of nine methylenes and a terminal methyl group (C-7′ to C-16′). The analysis of the NOESY spectrum allowed an *E* configuration to be established for the double bond based on the observation of a correlation between H-6′ and H_3-19′ (Figure 1). The structure of the acyl moiety therefore corresponds to (*E*)-2,2,4-trimethyl-3-oxohexadec-4-enoic acid, which represents a new 3-keto fatty acid. Thus, the planar structure of **1** was established as 1-*O*-[(*E*)-2′,2′,4′-trimethyl-3′-oxohexadec-4′-enoyl]glycero-3-*O*-4″-(*N*,*N*,*N*-trimethyl)-homoserine and given the name MDN-0104 (according to our proprietary compound database). ESIMS/MS experiments in positive ionization mode further confirmed the planar structure of this new monoacylglyceryltrimethylhomoserine (see Supporting Information). Likewise, the maximum absorbance observed at 239 nm in the UV (DAD) spectrum is in agreement with the expected value for the corresponding $\pi \rightarrow \pi^*$ transition of the α,β-unsaturated enone present in the structure.[28]

MDN-0104 (**1**) was isolated as an optically active material. We have assigned a tentative *S* absolute configuration to the two chiral centers of **1** based on biosynthetic arguments. Our hypothesis assumes that the new monoacylglyceryltrimethylhomoserine and the common diacylglyceryltrimethylhomoserines share the same biosynthetic pathway. The coexistence of the monoacylglyceryltrimethylhomoserines **2** and **3** with the related DGTS **4** in the green alga *Ulva fasciata*[8] supports such a hypothesis. The biosynthesis of DGTS (Scheme 1) has been characterized in phosphate-starved cells

Scheme 1. Biosynthetic Hypothesis for MDN-0104 (1)



of the purple bacterium *Rhodobacter sphaeroides*.[29] Heterologous expression experiments have identified the two enzyme systems, BtaA and BtaB, essential to the biosynthesis.[30] A parallel system, the *BTA1* gene, has also been characterized in the eukaryotic alga *Chlamydomonas reinhardtii* and consists of two domains, corresponding to the bacterial BtaA and BtaB proteins.[31] Furthermore, many fungal genomes encode an enzymatic system homogolous to BTA1 for DGTS biosynthesis, and fungi synthesize DGTS during phosphorus (P) limitation possibly as a common strategy among many

dx.doi.org/10.1021/np500577v | J. Nat. Prod. XXXX, XXX, XXX−XXX

eukaryotes for conserving P under P-limiting conditions.[32] However, the medium in our fermentation system supplied an excess of P for fungal growth, suggesting that such enzymes might be under a different regulatory control. On the other hand, the eukaryotic alga *C. reinhardtii* synthesizes DGTS to the exclusion of phosphatidylcholine regardless of P availability.[33] Due to the unusual nature of the fatty acid moiety in MDN-0104 (**1**), it seems reasonable that the parent DGTS from which **1** derives is not a mere structural substitute for phosphatidylcholine in the fungal membranes. Rather, betaine lipid **1** must play some ecological role[34] in *H. chenopodii*, which would explain its production in P-replete media. In any case, the parent DGTS is likely synthesized via an enzymatic system homologous to BTA1 (regardless of its regulatory control). In such a system, the first enzymatic domain (BtaA-like) transfers the 3-amino-3-carboxypropyl group of *S*-adenosyl-L-methionine to the 3-hydroxyl of a 1,2-diacyl-*sn*-glycerol to form the intermediate diacylglycerylhomoserine. The second enzyme domain (BtaB-like) transfers methyl groups from *S*-adenosyl-L-methionine in three successive steps to form the final product diacylglyceryl-*N,N,N*-trimethylhomoserine. The starting 1,2-*sn*-diacylglycerol in such a route has an *S* configuration in the glyceryl methine. On the other hand, the homoserine unit is transferred from *S*-adenosyl-L-methionine.[30] This renders an absolute *S* configuration for both chiral centers of the parent DGTS. Monoacylglyceryltrimethylhomoserines may derive from DGTS by regioselective enzymatic hydrolysis of the fatty acid at position *sn*-2 (Scheme 1). Such hydrolysis would be analogous to the selective hydrolysis of phosphatidylcholine catalyzed by phospholipase A_2 to render lysophosphatidylcholine.[35] It cannot be ruled out that a similar specialized lipase must be involved in the regioselective hydrolysis of DGTS. This would lead to the following lipid name for MDN-0104: 1-*O*-[(*E*)-2′,2′,4′-trimethyl-3′-oxohexadec-4′-enoyl]-*sn*-glycero-3-*O*-4″-(*N,N,N*-trimethyl)-L-homoserine.

MDN-0104 (**1**) exhibited activity against *P. falciparum Pf*3D7 using the previously described LDH growth inhibition *in vitro* assay[36] with an IC_{50} of 7.00 ± 0.25 μM. Chloroquine gave an IC_{50} of 5.5 nM when tested under the same conditions. The structural resemblance of MDN-0104 (**1**) to lysophosphatidylcholine may explain the observed bioactivity. It is possible that **1** induces a membrane disruption due to its analogous zwitterionic detergent structure.[37,38] Some biological effects of lysophosphatidylcholine may simply be due to its ability to diffuse readily into membranes, altering their curvature and indirectly affecting the properties of membrane proteins.[37] In fact, lysophosphatidylcholine has inspired the design of synthetic alkyl-lysophospholipids, such as miltefosine, which have recently found use as antiprotozoal drugs.[39] Even more interesting, hexadecyltrimethylammonium bromide, a detergent compound structurally similar to miltefosine, was recently found to exhibit potent *in vitro* activity against *P. falciparum* via an antimalarial mechanism based on the inhibition of the parasite choline kinase.[40] Thus, it cannot be ruled out that MDN-0104 displays antiplasmodial activity due to inhibition of the same enzyme.

Finally, we investigated whether other fungal species described in the genus *Heterospora* were able to produce MDN-0104 (**1**). To this end, a strain of the sister species *Heterospora dimorphospora* was chosen.[23] The epi-type strain employed (CBS 345.78) from *Chenopodium quinoa* was grown under the same conditions. LC-UV-MS analysis of its corresponding acetone extract revealed that this strain also produced compound **1**, although much less than *H. chenopodii*. Additionally, we found that the new betaine lipid was also produced by another strain of *H. chenopodii* (CBS 115.96) (data not shown). This result suggested that the species of *Heterospora* lineage could be a promising source of monoacylglyceryltrimethylhomoserines.

In conclusion, a new betaine lipid, MDN-0104 (**1**), that displays moderate antiplasmodial activity has been isolated from fermentation broths of *H. chenopodii*. It has also been found that *H. dimorphospora* produces this secondary metabolite, suggesting the potential of *Heterospora* species as a source of this type of betaine lipids. The novel compound **1** is the first monoacylglyceryltrimethylhomoserine ever reported in the Fungi, and its acyl moiety also represents a novel natural 3-keto fatty acid. Its discovery during a research program aimed at the identification of new antimalarial leads from MEDINA's natural product library of microbial extracts again confirms the value of microbial secondary metabolites as a potential source of novel chemical entities that could be further developed into new antimalarial drugs. The production of **1** in one among eight growth conditions is yet another example of the value of microfermentation arrays as a strategy for identifying nutritional conditions to produce novel compounds.[20]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotation was determined with a JASCO P-2000 polarimeter. IR spectrum was measured with a JASCO FT/IR-4100 spectrometer equipped with a PIKE MIRacle single reflection ATR accessory. NMR spectra were recorded on a Bruker Avance III spectrometer (500 and 125 MHz for ^1H and ^{13}C NMR, respectively) equipped with a 1.7 mm TCI MicroCryoProbe, using the signal of the residual solvent as internal reference (δ_H 3.31 and δ_C 49.0 ppm for CD_3OD). LC-UV-MS analysis was performed on an Agilent 1100 single quadrupole LC-MS system, using a Zorbax SB-C_8 column (2.1 × 30 mm, 5 μm), maintained at 40 °C and with a flow rate of 300 μL min^{-1}. Solvent A consisted of 10% acetronitrile and 90% water with 1.3 mM trifluoroacetic acid and ammonium formate, and solvent B was 90% acetronitrile and 10% water with 1.3 mM trifluoroacetic acid and ammonium formate. The gradient started at 10% B and went to 100% B in 6 min, was kept at 100% B for 2 min, and returned to 10% B for 2 min to initialize the system. Full diode array UV scans from 100 to 900 nm were collected in 4 nm steps at 0.25 s/scan. The eluting solvent was ionized using the standard Agilent 1100 electrospray ionization source adjusted to a drying gas flow of 11 L min^{-1} at 325 °C and a nebulizer pressure of 40 psig. The capillary voltage was set to 3500 V. Mass spectra were collected as full scans from 150 m/z to 1500 m/z, with one scan every 0.77 s, in both positive and negative modes. Database searching was performed using an in-house-developed application where the DAD (UV−vis) spectra, retention time, and positive and negative mass spectra of the samples are compared to the corresponding UV-LC-MS data of known microbial metabolites stored in the proprietary database (Fundación MEDINA reference library containing annotated metabolite data obtained under identical conditions to those for the samples under analysis; the library includes 380 fungal metabolites and 450 metabolites from bacteria and actinomycetes).[21,24−26] HRESIMS and MS/MS spectra were acquired using a Bruker maXis QTOF mass spectrometer coupled to the same HPLC system as described above. The mass spectrometer was operated in positive ESI mode. The instrumental parameters were 4 kV capillary voltage, drying gas flow of 11 L min^{-1} at 200 °C, and nebulizer pressure of 2.8 bar. TFA-Na cluster ions were used for mass calibration of the instrument prior to sample injection. Prerun calibration was by infusion with the same TFA-Na calibrant. Acetone used for extraction was of analytical grade. Solvents employed for isolation were of HPLC grade.

Strain and Fermentation. *Heterospora chenopodii* (CBS 109836) was purchased from the Centraalbureau voor Schimmelcultures (www.

dx.doi.org/10.1021/np500577v | J. Nat. Prod. XXXX, XXX, XXX−XXX

cbs.knaw.nl). The strategy and protocols for microfermentation of fungi on nutritional arrays have been described previously.[19,20,41] To scale up the microfermentation to 600 mL, 10 mycelial discs were used to inoculate 50 mL of SMYA (Difco neopeptone 10 g, maltose 40 g, Difco yeast extract 10 g, agar 4 g, distilled H_2O 1 L). After 7 days' incubation at 22 °C and 220 rpm, 0.3 mL aliquots of this culture were used to inoculate STP medium (sucrose 75 g, tomato paste 10 g, Difco malt extract 10 g, soy flour 1 g, $(NH_4)_2SO_4$ 1 g, KH_2PO_4 9 g, distilled H_2O 1 L) distributed among 60 × 10 mL in 40 mL flat-sided tissue culture tubes (TPP Techno Plastic Products AG) with 10 cm^2 growth area. The inclined TPP tubes were incubated statically at 22 °C and 70% relative humidity for 21 days. Fungal growth was removed from tubes and pooled before extraction.

Extraction and Isolation. The initial 1 mL microfermentations were extracted with an equal volume of acetone, and the acetone was removed by vacuum evaporation as described previously.[19,41] The scaled up fermentation broth (600 mL) was extracted with acetone (600 mL) under continuous shaking at 220 rpm for 1 h. The mycelium was then separated by centrifugation, and the supernatant (ca. 1.2 L) was concentrated to ca. 600 mL under a stream of nitrogen. This solution was loaded (with continuous 1:1 water dilution, discarding the flow-through) on a column packed with SP-207ss reversed-phase resin (brominated styrenic polymer, 65 g) previously equilibrated with water. The loaded column was further washed with water (ca. 1 L) and afterward eluted at 8 mL min^{-1} on an automatic flash-chromatography system (CombiFlash Rf, Teledyne Isco) using a linear gradient from 10% to 100% acetone in water (in 12.5 min) with a final 100% acetone step (for 15 min) collecting 11 fractions of 20 mL. Fractions were concentrated to dryness on a centrifugal evaporator, and fraction 6, containing the active compound, was further purified by reversed-phase semipreparative HPLC (Agilent Zorbax SB-C_8, 9.4 × 250 mm, 7 μm; 3.6 mL min^{-1}, UV detection at 210 nm) with a linear gradient of water−CH_3CN from 5% to 100% CH_3CN over 37 min to yield compound **1** (7.7 mg) eluting at 31 min.

Compound ***1*** *(1-O-[(E)-2′,2′,4′-trimethyl-3′-oxohexadec-4′-enoyl]-sn-glycero-3-O-4″-(N,N,N-trimethyl)-L-homoserine):* white, amorphous solid; $[\alpha]^{20}_D$ +9.4 (*c* 0.23, MeOH); IR (ATR) ν_{max} 3358, 2955, 2923, 2854, 1735, 1666, 1627, 1465, 1388, 1361, 1269, 1123, 1042 cm^{-1}; for ^1H and ^{13}C NMR data see Table 1; HRESIMS *m*/*z* 528.3907 $[M + H]^+$ (calcd for $C_{29}H_{54}NO_7$, 528.3895); MS/MS (see Supporting Information).

Biological Activity. Parasites of the *P. falciparum* strain 3D7 were grown in fresh group 0 positive human erythrocytes, obtained from the Centro Regional de Transfusión Sanguínea-SAS (Granada, Spain), and suspended at 5% hematocrit in RPMI 1640 containing 2% human serum, 0.2% $NaHCO_3$, 0.5% Albumax II, 150 μM hypoxanthine, and 12.5 μg/mL gentamicin. Flasks were incubated at 37 °C, under a 5% CO_2 and 95% air mixture. Stock cultures were synchronized with 5% sorbitol, and 96 h later parasites were mostly late ring stages and early trophozoites. The stock culture was then diluted with complete medium and nonparasitized erythrocytes to yield a hematocrit of 2% and a parasitemia of 0.25%. The extracts, fractions, and pure compounds were evaluated in 384-well plates. Each plate also included negative (no additions) and positive controls with 100 nM chloroquine. Parasite growth inhibition assays and 50% inhibitory concentration (IC_{50}) determinations were measured using the LDH assay as previously described.[36]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR, HR-MS/MS, and DAD (UV−vis) spectra for compound **1** and photographs of the microorganism in culture are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*(I. Pérez-Victoria) Tel: +34 958993965, ext 7017. Fax: +34 958846710. E-mail: ignacio.perez-victoria@medinaandalucia.es.

*(F. Reyes) Tel: +34 958993965, ext 7006. Fax: +34 958846710. E-mail: fernando.reyes@medinaandalucia.es.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research has been supported by grants from the Junta de Andalucía (BIO-199, BIO-023, BIO-024, BIO-025, P09-CVI-5367), the VI PN de I+D+I 2008-2011, ISCIII-Subdirección General de Redes y Centros de Investigación Cooperativa (RICET FIS Network: RD12/0018/0017 and RD12/0018/0005), and a Marie Curie Career Integration Grant (I.P.-V.) [PCIG-GA-2011-293762]. We thank C. Moreno, F. Muñoz, L. Rodríguez, and J. Cantizani for technical assistance and suggestions. The polarimeter, HPLC, IR, and NMR equipment, and plate reader used in this work were purchased via grants for scientific and technological infrastructures from the Ministerio de Ciencia e Innovación [Grants No. PCT-010000-2010-4 (NMR), INP-2011-0016-PCT-010000-ACT6 (polarimeter, HPLC, and IR), and PCT-01000-ACT7, 2011-13 (plate reader)].

■ REFERENCES

(1) Vaskovsky, V. E.; Khotimchenko, S. V.; Boolugh, E. M. *Phytochemistry* **1998**, *47*, 755−760.
(2) Rozentsvet, O. A.; Dembitsky, V. M.; Saksonov, S. V. *Phytochemistry* **2000**, *54*, 401−407.
(3) Künzler, K.; Eichenberger, W. *Phytochemistry* **1997**, *46*, 883−892.
(4) Kato, M.; Sakai, M.; Adachi, K.; Ikemoto, H.; Sano, H. *Phytochemistry* **1996**, *42*, 1341−1345.
(5) Geiger, O.; González-Silva, N.; López-Lara, I. M.; Sohlenkamp, C. *Prog. Lipid Res.* **2010**, *49*, 46−60.
(6) Eichenberger, W.; Araki, S.; Müller, D. G. *Phytochemistry* **1993**, *34*, 1323−1333.
(7) Dembitsky, V. M. *Prog. Lipid Res.* **1996**, *35*, 1−51.
(8) Siddhanta, A. K.; Goswami, A. M.; Ramavat, B. K.; Achari, B. *J. Indian Chem. Soc.* **2002**, *79*, 843−845.
(9) Shetty, P. *Nature* **2012**, *484*, S14−S15.
(10) http://www.who.int/malaria/publications/world_malaria_report_2013/en/index.html.
(11) Wells, T. N. C. *Malaria J.* **2011**, *10* (Suppl 1), S3.
(12) Flannery, E. L.; Chatterjee, A. K.; Winzeler, E. A. *Nat. Rev. Microbiol.* **2013**, *11*, 849−862.
(13) Eisenstein, M. *Nature* **2012**, *484*, S16−S18.
(14) Sanchez, S.; Guzmán-Trampe, S.; Ávalos, M.; Ruiz, B.; Rodríguez-Sanoja, R.; Jiménez-Estrada, M. Microbial Natural Products. In *Natural Products in Chemical Biology*; Civjan, N., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, 2012; pp 65−108.
(15) Pearce, C.; Eckard, P.; Gruen-Wollny, I.; Hansske, F. G. Microorganisms: Their Role in the Discovery and Development of Medicines. In *Natural Product Chemistry for Drug Discovery*; Buss, A. D.; Butler, M. S., Eds.; The Royal Society of Chemistry: Cambridge, 2010; pp 215−241.
(16) Guantai, E.; Chibale, K. *Malaria J.* **2011**, *10* (Suppl1), S2.
(17) Xu, D.; Ondeyka, J.; Harris, G. H.; Zink, D.; Kahn, J. N.; Wang, H.; Bills, G.; Platas, G.; Wang, W.; Szewczak, A. A.; Liberator, P.; Roemer, T.; Singh, S. B. *J. Nat. Prod.* **2011**, *74*, 1721−1730.
(18) Pérez-Victoria, I.; Martín, J.; González-Menéndez, V.; de Pedro, N.; El Aouad, N.; Ortiz-López, F. J.; Tormo, J. R.; Platas, G.; Vicente, F.; Bills, G. F.; Genilloud, O.; Goetz, M. A.; Reyes, F. *J. Nat. Prod.* **2012**, *75*, 1210−1214.
(19) Ondeyka, J.; Harris, G.; Zink, D.; Basilio, A.; Vicente, F.; Bills, G.; Platas, G.; Collado, J.; González, A.; Cruz, M. d. l.; Martin, J.; Kahn, J. N.; Galuska, S.; Giacobbe, R.; Abruzzo, G.; Hickey, E.; Liberator, P.; Jiang, B.; Xu, D.; Roemer, T.; Singh, S. B. *J. Nat. Prod.* **2008**, *72*, 136−141.

(20) Bills, G. F.; Platas, G.; Fillola, A.; Jiménez, M. R.; Collado, J.; Vicente, F.; Martín, J.; González, A.; Bur-Zimmermann, J.; Tormo, J. R.; Peláez, F. *J. Appl. Microbiol.* **2008**, *104*, 1644−1658.
(21) Bills, G. F.; Martín, J.; Collado, J.; Platas, G.; Overy, D.; Tormo, J. R.; Vicente, F.; Verkleij, G.; Crous, P. *SIMB News* **2009**, *59*, 133−147.
(22) van der Aa, H. A.; van Kesteren, H. A. *Persoonia* **1980**, *10*, 542−542.
(23) de Gruyter, H.; Woudenberg, J. H. C.; Aveskamp, M. M.; Verkley, G. J. M.; Groenewald, J. Z.; Crous, P. W. *Stud. Mycol.* **2013**, *75*, 1−36.
(24) Zink, D.; Dufresne, C.; Liesch, J.; Martín, J., Identification/dereplication of natural products by LC−UV−MS. Spectral search parameters. Presented at the Small Molecule Science Conference (COSMOS), Bristol, Rhode Island, August 8−11, 2005.
(25) Zink, D.; Dufresne, C.; Liesch, J.; Martín, J., Automated LC−MS analysis of natural products: extraction of UV, MS and retention time data for component identification and characterization. In *Proceedings of the 50th ASMS Conference on Mass Spectrometry and Allied Topics*, Orlando, Florida, June 2−6, 2002.
(26) Vicente, F.; Basilio, A.; Platas, G.; Collado, J.; Bills, G. F.; González Del Val, A.; Martín, J.; Tormo, J. R.; Harris, G. H.; Zink, D. L.; Justice, M.; Nielsen Kahn, J.; Peláez, F. *Mycol. Res.* **2009**, *113*, 754−770.
(27) *Dictionary of Natural Products on DVD*, version 22.2; Taylor & Francis/CRC Press: London, UK, 2013.
(28) Woodward, R. B. *J. Am. Chem. Soc.* **1941**, *63*, 1123−1126.
(29) Hofmann, M.; Eichenberger, W. *J. Bacteriol.* **1996**, *178*, 6140−6144.
(30) Riekhof, W. R.; Andre, C.; Benning, C. *Arch. Biochem. Biophys.* **2005**, *441*, 96−105.
(31) Riekhof, W. R.; Sears, B. B.; Benning, C. *Eukaryot. Cell* **2005**, *4*, 242−252.
(32) Riekhof, W. R.; Naik, S.; Bertrand, H.; Benning, C.; Voelker, D. R. *Eukaryot. Cell* **2014**, *13*, 749−757.
(33) Giroud, C.; Gerber, A.; Eichenberger, W. *Plant Cell Physiol.* **1988**, *29*, 587−595.
(34) O'Brien, J.; Wright, G. D. *Curr. Opin Biotechnol.* **2011**, *22*, 552−558.
(35) Burke, J.; Dennis, E. *Cardiovasc. Drugs Ther.* **2009**, *23*, 49−59.
(36) Gamo, F.-J.; Sanz, L. M.; Vidal, J.; de Cozar, C.; Alvarez, E.; Lavandera, J.-L.; Vanderwall, D. E.; Green, D. V. S.; Kumar, V.; Hasan, S.; Brown, J. R.; Peishoff, C. E.; Cardon, L. R.; Garcia-Bustos, J. F. *Nature* **2010**, *465*, 305−310.
(37) Weltzien, H. U. *Biochim. Biophys. Acta, Biomembr.* **1979**, *559*, 259−287.
(38) Arouri, A.; Mouritsen, O. G. *Prog. Lipid Res.* **2013**, *52*, 130−140.
(39) Dorlo, T. P. C.; Balasegaram, M.; Beijnen, J. H.; de Vries, P. J. *J. Antimicrob. Chemother.* **2012**, *67*, 2576−2597.
(40) Choubey, V.; Maity, P.; Guha, M.; Kumar, S.; Srivastava, K.; Puri, S. K.; Bandyopadhyay, U. *Antimicrob. Agents Chemother.* **2007**, *51*, 696−706.
(41) Duetz, W.; Chase, M.; Bills, G. F. Miniaturization of Fermentations. In *Manual of Industrial Microbiology and Biotechnology*, 3rd ed.; Baltz, R. H. D., Julian, E.; Demain, A. L., Eds.; ASM Press: Washington, D.C., 2010; pp 99−116.