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Antiplasmodial Compounds from *Cochlospermum tinctorium*

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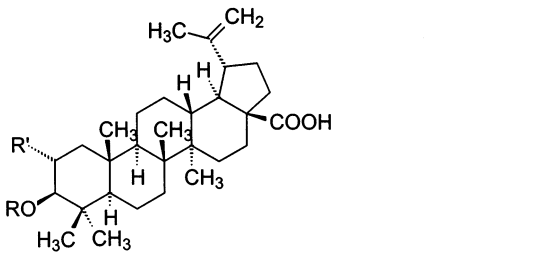
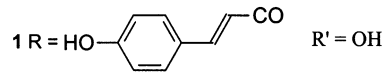
Fractionation of an ethanol extract of roots of *Cochlospermum tinctorium* afforded five compounds: 3-*O*-*E*-*p*-coumaroylalphitolic acid (**1**), cochloxanthin (**2**), dihydrocochloxanthin (**3**), alphitolic acid (**4**), and 1-hydroxytetradecan-3-one (**5**). This is the first example of a 1-hydroxyalkan-3-one obtained from plant material after gentle workup. The antiplasmodial activities of the compounds were determined, and the IC_{50} value of 3-*O*-*E*-*p*-coumaroylalphitolic acid was 2.3 μM.

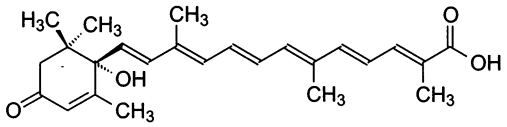
Malaria continues to be one of the largest health problems in the world, accounting for the death of more than two million people each year, the majority of whom are African children younger than five years.[1] An increasing development of resistance against the clinically used drugs increases the burden of the disease.[2] A decoction prepared from the comminuted roots of *Cochlospermum tinctorium* A. Rich. (Cochlospermaceae) purchased in plastic bags is used as a drug against malaria in Burkina Faso. Previous studies on the oil obtained by hydrodistillation of the leaves of *C. tinctorium* have revealed a significant antiplasmodial activity of the oil. A number of aliphatic ketones including 1-hydroxytetradecan-3-one, aldehydes, esters, and some terpenoids have by GC−MS been shown to be present in the oil, but the activity has not been related to any of the constituents.[3] Extracts of the roots show a significantly higher antiplasmodial activity than that reported for the essential oil.[4] Consequently the present study was undertaken in order to characterize some of the nonvolatile antiplasmodial compounds.

Whereas an aqueous decoction of the roots showed no antiplasmodial activity in vitro, an ethanol extract, in agreement with previous findings, showed a pronounced activity (1−2 μg/mL).[4] Partitioning between water and dichloromethane of the ethanol extract revealed that the major activity was found in the organic layer. Fractionation of the dichloromethane phase afforded five compounds: 3-*O*-*E*-*p*-coumaroylalphitolic acid (**1**),[5] cochloxanthin (**2**),[5] dihydrocochloxanthin (**3**),[5] alphitolic acid (**4**),[5] and 1-hydroxytetradecan-3-one (**5**), which previously has been characterized only by GC−MS[6] and by MS in a mixture with 1-hydroxyhexadecan-3-one.[5] Consequently **5** has never been thoroughly characterized. A fractionation aimed at isolation of **5** using 2,4-dinitrophenylhydrazine for visualization revealed the presence of approximately 0.1% of **5** in the dried plant material. 1-Hydroxyhexadecan-16-one was not found in this plant material. Compound **5** was characterized by MS and NMR spectroscopy. A total synthesis of **5** (Scheme 1) was performed in order to get a colorless sample, since the product isolated from the natural source was a reddish oil, probably contaminated with trace amounts of apocarotenoids. The NMR spectra of the natural product and the synthetic product were identical. Only a few 1-hydroxyalkan-3-ones have previously been reported. Some examples are 1-hydroxyhexadecan-3-one, which was found in the steam distillate from roots of *C. tinctorium* and characterized by the mass spectrum,[7] 1-hydroxyundecan-3-one, 1-hydroxytridecan-3-one, and 1-hydroxyheptadecan-3-one. The latter three all have been detected in the essential oil from leaves of *C. tinctorium* and *C. planchonii* by GC−MS.[3] 1-Hydroxyoctan-3-one has been detected after hydrolysis of a fraction containing glycosides from *Carica pubescens* fruit pulp.[8] This is the first report of a 1-hydroxyalkan-3-one obtained in a pure state after a gentle workup of a plant extract.





4 R = H, R' = OH
6 R= H, R' = H



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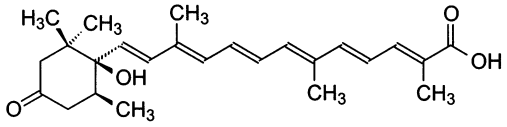

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The antiplasmodial activities of **1**, **4**, and **5** are given in Table 1. Poor solubility of **2** and **3** prevented measurement

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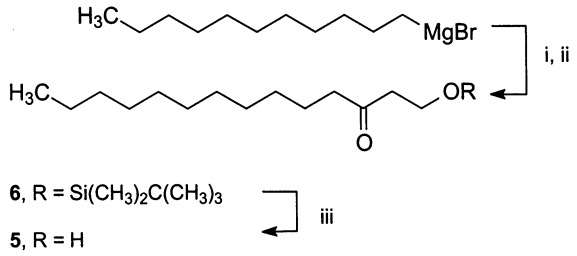
Scheme 1. Synthesis of 1-Hydroxytetradecan-3-one



(i) $(CH_3)_3CSi(CH_3)_2O(CH_2)_2CN$; (ii) Aqueous HCl; (iii) Methanolic HCl

Table 1. Antiplasmodial Activity and Activity against Phytohaemagglutinin A-Activated Human Lymphocytes of Compound **1**, **4**, and **5**[a]

	IC_{50} (μM)		
compound	(3d7)	(Dd2)	PHA
1	2.3 ± 1.1	3.8 ± 1.9	43 ± 12
4	35		
5	68 ± 19		
chloroquine	(22 ± 9) × 10^{-3}	(172 ± 25) × 10^{-3}	

[a] The *P. falciparum* strain (3d7) is chloroquine sensitive, whereas (Dd2) is chloroquine resistant. The column PHA shows phytohaemagglutinin A-provoked lymphocyte proliferation. Compound **1** was tested in triplicate in three experiments carried out on different days. Compound **4** was tested once in triplicate. Synthetic compound **5** was tested in triplicate in two experiments carried out on different days. Chloroquine was tested in triplicate in three experiments carried out on different days.

of their biological activities. Compound **1** showed an interesting antiplasmodial activity, whereas the activity toward human lymphocytes was only moderate, indicating some selectivity. The activity of the coumaroyl derivative (**1**) was significantly higher than that of alphitolic acid (**4**) and the related betulinic acid (**6**),[9] which also has been found in the plant.[5] These findings are similar to the activities of chalcones (e.g., licochalcone A), which also contain a cinnamoyl residue.[10]

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini at 300 and 75 MHz, respectively. The optical rotation was measured on a Perkin-Elmer 241 polarimeter. TLC: Merck silica gel 60 $PF_{254+360}$. VLC: Merck silica gel for preparative TLC.

Biological Material. Rhizomes of *Cochlospermum tinctorium* were collected in Tita Central-West of Burkina Faso in April 2000 and identified by Dr. Ouétien Bognounou. A voucher specimen (KMD 1) has been deposited at the Royal Danish School of Pharmacy, Department of Medicinal Chemistry.

Antiplasmodial Assay. Assays for antiplasmodial activity using a chloroquine-sensitive (3D7) and -resistant (Dd2) *Plasmodium falciparum* strain were performed as described in detail elsewhere.[11]

Lymphocyte Proliferation Assay. The effects of the compounds on phytohaemmagglutinin A-provoked proliferation of human lymphocytes were assessed by monitoring the uptake of radiolabeled tymidine as previously described.[12]

Extraction and Isolation. The residue of an ethanolic extract of 0.5 kg of comminuted air-dried roots was suspended in water (500 mL) and extracted with petroleum ether, CH_2Cl_2, and EtOAc (500 mL of each). The three phases were concentrated in vacuo. The residue of the CH_2Cl_2 phase (8.03 g) showed the highest activity and was chromatographed on silica gel 60 (Merck, 800 g) using 500 mL of the eluents CH_2Cl_2, CH_2Cl_2−EtOAc (19:1, 14:1, 9:1, 1:1, 1:1), 2000 mL of CH_2Cl_2−EtOAc (1:2), and 500 mL of EtOAc. The first part of the fraction (290 mg) eluting with CH_2Cl_2−EtOAc (1:1) was chromatographed by HPLC (Phenomenex C_{18}, 5 μm, 150 × 21.2 mm column) using MeCN−H_2O (85:15, flow 9.0 mL/min) to give **1** (3.3 mg of an amorphous powder, retention time 6 min, $[\alpha]^{25}_D$ −36° (*c* 0.4, pyridine [lit.][5] $[\alpha]^{25}_D$ −33°) and **5** (2.3 mg of an oil, retention time 8 min). The second part of the fraction (530 mg) eluting with CH_2Cl_2−EtOAc (1:2) was chromatographed by HPLC (Supelco Discovery C_{18}, 5 μm, 150 × 21.2 mm column) using MeCN−H_2O (85:15, flow 9.0 mL/min) to give **2** (3.0 mg, retention time 16 min, $[\alpha]^{25}_D$ +1.4° × 10^3 (*c* 0.4, $CHCl_3$ [lit.][5] $[\alpha]^{25}_D$ +1.3° × 10^2) and **3** (10.8 mg, retention time 18 min). The fraction eluting with EtOAc was chromatographed over RP-8 (Merck, 35 g) using MeOH−H_2O (9:1, 19:2, 21:2, 11:1, 14:1, 19:1, 29:1; 300 mL of each). The fraction eluting with MeOH−H_2O (19:2, 180 mg) was chromatographed on silica gel 60 (Merck 35 g) using CH_2Cl_2−EtOAc (8:2, 600 mL; 7:3, 400 mL; 6:4, 400 mL; 5:5, 400 mL; 4:6, 200 mL; 3:7, 200 mL) to give **4** (15 mg of an amorphous powder, $[\alpha]^{25}_D$ −0.24° (*c* 0.1, $CHCl_3$ [lit.][5] $[\alpha]^{25}_D$ −4°).

1-Hydroxytetradecan-3-one (5): reddish oil; ^{1}H NMR ($CDCl_3$, 300 MHz) 3.84 (2H, q *J* = 5.5 Hz, H-1), 2.67 (2H, t *J* = 5.5 Hz, H-2), 2.49 (1H, t *J* = 5.5 Hz, OH), 2.43 (2H, q *J* = 7.6 Hz, H-4), 1.57 (2H, m, H-5), 2.25 (16H, m, H-6−H-13), 0.88 (3H, t *J* = 5.5 Hz, H-14); ^{13}C NMR ($CDCl_3$, 75 MHz) 57.99 (C-1), 44.33 (C-2), 212.56 (C-3), 43.49 (C-4), 23.71 (C-5), 29.19, 29.33, 29.39, 29.46, 29.61 (C-6−C-11) 31.97 (C-12) 22.74 (C-13), 14.16 (C-14); FAB$^+$HRMS 229.2154; calcd for $C_{14}H_{29}O_2$ 229.2167.

2,4-Dinitrophyenylhydrazine (DNP)-Guided Isolation of 1-Hydroxytetradecan-3-one (5). Comminuted rhizomes (105 g) were extracted with CH_2Cl_2 to give 4.22 g of a crude extract. The extract was chromatographed by VLC, silica gel 60 (Merck 60 g), using 100 mL of CH_2Cl_2, CH_2Cl_2−EtOAc (19:1, 14:1, 4.1, 1:1), EtOAc, and EtOAc−MeOH (2:1, 1:1, 1:2). The fractions eluted by EtOAc and EtOAc−MeOH (2:1, 1:1) were visualized with DNP and were combined and concentrated to give compound **5** (0.14 g). Compound **5** was further chromatographed on silica gel 60 (Merck 30 g) using 4:1 CH_2Cl_2−EtOAc as an eluent, and the fractions were visualized with DNP (170−260 mL), combined, and concentrated to give 0.12 g. Final purification was obtained by chromatography on silica gel using CH_2Cl_2−EtOAc (4:1) as an eluent to give compound **5** (106 mg).

Synthesis of 1-*tert*-Butyldimethylsilyloxytetradecan-3-one (6). Undecanemagnesium bromide (1.95 mol in 20 mL of dry ether) was added dropwise under nitrogen to 3-*tert*-butyldimethylsilyloxypropionitrile (0.317 mol) at 0 °C. The solution was stirred overnight at room temperature. Ice (50 g) was added to the solution, and the pH was adjusted to 5 with concentrated hydrochloric acid. The solution was stirred for 2 h and the organic layer isolated. The aqueous layer was extracted with ether (50 mL). The combined organic phases were dried ($MgSO_4$) and concentrated. The residue was chromatographed over silica gel using CH_2Cl_2−petroleum ether (bp 45−60 °C) (1:1) as an eluent to give 0.119 g (19.5%) of 1-*tert*-butyldimethylsilyloxytetradecan-3-one as a colorless oil.

Synthesis of 1-Hydroxytetradecan-3-one (5). The crude 1-*tert*-butyldimethylsilyloxytetradecan-3-one (0.119 g) was dissolved in methanolic HCl (0.4 M, 2.5 mL) and left at room temperature for 1 h. After concentration in vacuo the residue was chromatographed over silica gel 60 (Merck, 2.7 g) using CH_2Cl_2 as an eluent to give 1-hydroxytetradecan-3-one (0.027 g, 23%). The NMR spectra of the synthesized **2** were identical to those of the natural product.

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References and Notes

(1) Kilma, W. L. *Am. J. Trop. Med. Hyg.* **2001**, *64*, iii−iii.
(2) Trape, J. F. *Am. J. Trop. Med. Hyg.* **2001**, *64*, 12−17.

(3) Benoit-Vical, F.; Valentin, A.; Mallie, M.; Bastide, J.-M.; Bessière, J.-M. *Planta Med.* **1999**, *65*, 378−381.
(4) Benoit, F.; Valentin, A.; Pélissier, Y.; Marion, C.; Dakuyo, Z.; Mallié, M.; Bastide, J. M. *Trans. R. Soc. Trop. Med. Hyg.* **1995**, *89*, 217−218.
(5) Luschmann, K. *Isolierung und Strukturermittlung von Inhaltsstoffen aus den westafrikanischen Arzneipflanzen Cochlospermum planchonii und Cochlospermum tinctorium. Neue Naturstoffe der Acetogenin-bzw. Apocarotinoid-reihe.* Ph.D. Thesis, Friedrich-Alexander-Universität Erlangen Nürnberg, 1989, pp 1−271.
(6) Benoit-Vical, F.; Valentin, A.; Mallié, M. *J. Essent. Oil. Res.* **2001**, *13*, 65−67.
(7) Diallo, B.; Vanhaelen-Fastre, R.; Vanhaelen, M. *Phytochemistry* **1991**, *30*, 4153−4156.
(8) Krajewski, D.; Duque, C.; Schreiber, P. *Phytochemistry* **1997**, *45*, 1627−1631.
(9) Steele, J. C. P.; Warhurst, D. C.; Kirby, G. C.; Simmonds, M. S. J. *Phytother. Res.* **1999**, *13*, 115−119.
(10) Chen, M.; Theander, T. G.; Christensen, S. B.; Hviid, L.; Zhai, L.; Kharazmi, A. *Antimicrob. Agents Chemother.* **1994**, *38*, 1470−1475.
(11) Ziegler, H.; Stærk, D.; Christensen, J.; Hviid, L.; Hägerstrand, H.; Jaroszewski, J. W. *Antimicrob. Agents Chemother.* **2001**, *46*, 1441−1446.
(12) Bygbjerg, I.; Flachs, H. *Trans. R. Soc. Trop. Med. Hyg.* **1986**, *80*, 231−235.

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