Absolute Configuration and Antiprotozoal Activity of Minquartynoic Acid

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Minquartynoic acid (**1**) was isolated as an antimalarial and antileishmanial constituent of the Peruvian tree *Minquartia guianensis* and its absolute configuration at C-17 established to be (+)-*S* through conversion to the known (+)-(*S*)-17-hydroxystearic acid (**2**) and confirmed using Mosher's method.

Minquartia guianensis has been used in South American traditional medicine as an anthelmintic[1] and against lung cancer and tuberculosis.[2] During ethnobotanical fieldwork in the Department of Loreto in Peru it was found that *M. guianensis* is widely used for the treatment of malaria and leishmaniasis in the area. Bioassay guided fractionation of an extract of *M. guianensis* revealed that the antiparasitic activity was associated with the main constituent of the extract, which was identified as minquartynoic acid by its NMR and physical data.[2] Minquartynoic acid is present in extraordinarily large amounts in the bark (2−3% of the dry weight). Because of the acidic nature of **1**, the chromatographic separation was performed on dihydrogenphosphate-impregnated silica gel, which eliminated tailing and resulted in better separation. Several attempts to make crystalline derivatives of **1** for X-ray crystallographic determination of the absolute configuration at C-17 were unsuccessful. Instead, **1** was converted to 17-hydroxystearic acid (**2**) by hydrogenation, and the optical rotation compared *R*- and *S*-isomers.[3,4] The *S*-configuration of **1** was confirmed, using Mosher's method,[5] by esterification of the methyl ester **3** with racemic and (−)-(*R*)-*O*-methylmandelic acid. Attempts to esterify the methyl ester of the acetylenic alcohol (**1**) itself, by the DCC-DMAP method, lead only to degradation of the starting material. Dale and Mosher[5] have compared the spectra of *R*- and *S*-*O*-methylmandelic acid esterified with *R*-methylcarbinols. In all cases the signal of the methyl group in the spectrum of the *R,R*-diasteromer was found 0.05−0.15 ppm more downfield than the corresponding signal in the spectrum of the *S,R*-isomer. Analogously the methyl signal in the *S,S*-ester must be found 0.05−0.15 ppm more downfield than the corresponding signal in the spectrum of the *R,S*-isomer. In the case of **3**, obtained from **1**, the methyl signal of the ester of *R*-*O*-methylmandelic acid was found at 1.08 ppm, and the methyl signal of the *S*-ester was observed at 1.21 ppm.

The two conformations shown in Figure 1 depict the preferred conformation of the esters of *S*-**3** with *R*- and *S*-*O*-methoxymandelic acid, respectively, if it is assumed that the C−O bond of the methoxy group is aligned with the C═O double bond of the carbonyl group.[5] Figure 1 reveals that the terminal methyl group will be juxtaposed with the α-phenyl in the ester of the *R*-*O*-methylmandelic acid, whereas the methylene chain will be juxtaposed with the α-phenyl in the *S*-ester. Consequently the signal of the terminal methyl group would be found upfield in the spectrum of the ester with *R*-mandelic acid relative to the signal in the *S*-ester. The reverse would be the case in the spectra of *R*-**3** with *R*- and *S*-*O*-methylmandelic acid. Since the signal of the terminal methyl group in the ester of 17-hydroxystearic acid prepared from minquartynoic acid is found at higher field in the ester with *S*-*O*-methylmandelic acid, 17-hydroxystearic acid and minquartynoic acid must possess the *S*-configuration.

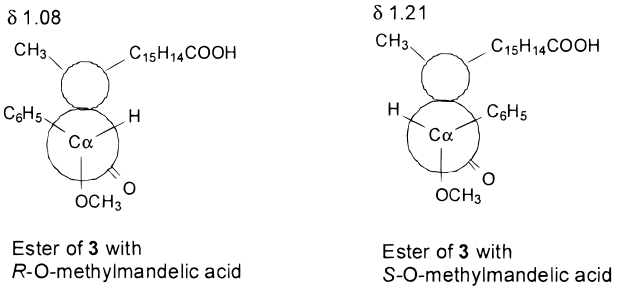
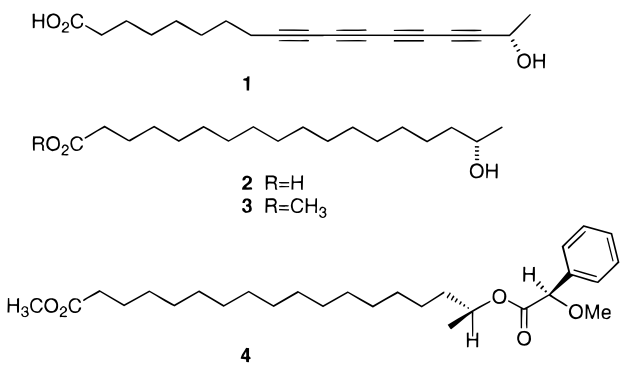


Figure 1. Preferred conformations of the Mosher[5] esters of **3**.



Minquartynoic acid (**1**) shows moderate in vitro activity against *Plasmodium falciparum* and *Leishmania major*, but the inhibition of phytohaemagglutinin A-induced proliferation of human lymphocytes suggests that the activity is due to the compound being generally cytotoxic (Table 1).

Experimental Section

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

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Table 1. IC_{50} Values (μg/mL) against *P. falciparum* and *L. major* and Inhibition of Phytohaemagglutinin A-Induced Proliferation of Human Lymphocytes[a]

	P. falciparum	L. major	PHA
1	3.0 ± 0.5	1.4 ± 0.1	5.3 ± 2.0
chloroquine	0.01 ± 0.001		
pentostam		67 ± 13	

Phosphate Impregnated Silica Gel. Two volumes of acetone were added to a well-stirred suspension of TLC grade Si gel (Merck 60H without binder) (200 g) in water (200 mL) containing NaH_2PO_4 (5 wt % of the Si gel). The resulting suspension was filtered, and the impregnated material washed twice with acetone and air-dried in a large dish. The adsorbent was activated by oven drying overnight at 110 °C.

Biological Material. *M. guianensis* was collected by A. G. Huansi in the Jenaro region of northern Peru, identified by Dr. Lars Peter Kvist, and a voucher specimen (G. 014) is kept in the herbarium at Universidad Nacional de la Amazonia Peruana, Iquitos, Peru.

Extraction an Isolation of Minquartynoic Acid (1). The ground, dried cortex (110 g) was macerated with a mixture of dichloromethane−methanol (1:1) (4×, 800 mL) and evaporated to give a crude extract (9.9 g). The crude extract was fractionated by VLC[6,7] on phosphate-impregnated Si gel (240 g) and eluted in steps with CH_2Cl_2−MeOH (25 mL fractions) (100:0, 100 mL; 95:5, 100 mL; 90:10, 200 mL; 85:15, 100 mL). **1** was eluted with CH_2Cl_2−MeOH (90:10, F10−F16, 2.4 g), and recrystallization from $CHCl_3$ furnished the pure acid **1** (2.1 g): mp 94−95 °C, $[\alpha]^{23}_D = -30°$ ($c = 0.2$, MeOH; lit.: −29°).[2]

Absolute Configuration of 1. Minquartynoic acid (100 mg) was dissolved in EtOAc (20 mL). Pd/C (10 mg) was added and the mixture hydrogenated at atmospheric pressure (30 min). The catalyst was removed by filtration through Celite and the solvent evaporated. The product was purified by VLC on phosphate-impregnated Si gel (20 g) eluted with CH_2Cl_2−MeOH (90:10), which upon evaporation gave pure 17-(*S*)-hydroxystearic acid: mp 79−80 °C, $[\alpha]^{23}_D = +4.8°$ ($c = 1.6$, AcOH; lit.: $[\alpha]^{22}_D = +4.4°$).[4]

Methyl Ester of 2. To a solution of 17-(*S*)-hydroxystearic acid (200 mg) dissolved in methanol (10 mL) was added 2 drops of TMSCl and the mixture stirred for 2 h. The volatiles were removed by evaporation, furnishing the ester (**3**), which was used without further purification.

(−)-(*R*)-*O*-Methylmandelic Ester (4). To a solution of **3** (55 mg, 0.18 mmol), (−)-(*R*)-*O*-methylmandelic acid (33 mg, 0.2 mmol), and DCC (40 mg, 0.19 mmol) in dry CH_2Cl_2 (5 mL) was added DMAP (2 mg, 0.02 mmol) and the mixture stirred for 2 h. The mixture was filtered through a short plug of Si gel, which was further eluted with CH_2Cl_2 (5 mL). The solvent was removed by evaporation, leaving mandelate (**4**) as an oil, which was used directly for the NMR experiments. The same procedure was followed with the racemic *O*-methylmandelic acid.

Antiplasmodial Assay. A modification of Desjardin's radioisotope method for measuring parasite growth was adopted for the assay.[8] A chloroquine-susceptible strain of *Plasmodium falciparum* (3D7) was incubated for 24 h in standard RPMI medium supplemented with 5% human serum. ^{3}H-hypoxanthine was added, and the cells were harvested after incubation (24 h). The compounds were dissolved in dimethyl sulfoxide and added in concentrations ranging from 100 to 0.1 μg/mL. The dimethyl sulfoxide concentration was kept below 1% in the medium, and chloroquine was used as positive control.

Antileishmanial Assay. A World Health Organization reference vaccine strain of *Leishmania major*, originally isolated from a patient in Iran, was used. Promastigotes were cultured at 26 °C as previously described.[9] The compounds were tested in concentrations ranging from 100 to 0.1 μg/mL, and pentostam was used as positive control.

Lymphocyte Proliferation Assay. The effects of the compounds on the phytohaemagglutinintion of human lymphocytes was assessed by monitoring the uptake of radiolabeled thymidine as previously described.[10] The compounds were tested in concentrations ranging from 100 to 0.1 μg/mL.

References and Notes

(1) Iglesias, G. *Hierbas Medicinales de los Quichuas del Napo, Collection Nuchanchic Unanche*; Ediciones Abaya-Yala: Quito, Equador, 1985.
(2) Marles, R. J.; Farnsworth, N. R.; Neill, D. A. *J. Nat. Prod.* **1989**, *52*, 261−266.
(3) Hammerström, S.; Hamberg, M. *Anal. Biochem.* **1973**, *52*, 169−179.
(4) Gorin, P. A. J.; Spencer, J. F. T.; Tulloch, A. P. *Can. J. Chem.* **1961**, *39*, 846−855.
(5) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519.
(6) Coll, J. C.; Bowden, B. F. *J. Nat. Prod.* **1986**, *49*, 934−936.
(7) Pelletier, S. W.; Chokshi, H. P.; Desai, H. K. *J. Nat. Prod.* **1986**, *49*, 892−900.
(8) Desjardins, R. E.; Canfield, R. E.; Hayness, C. J.; Chulay, J. D. *Antimicrob. Agents Chemther.* **1979**, *16*, 710−718.
(9) Chen, M.; Christensen, S. B.; Blom, J.; Lemmich, E.; Nadelmann, L.; Fich, K.; Theander, T. G.; Kharazmi, A. *Antimicrob. Agents Chemother.* **1993**, *37*, 2550−2556.
(10) Bygbjerg, I. C.; Theander, T. G.; Andersen, B. J.; Flachs, H.; Jepsen, S.; Larsen, P. B. *Trop. Med. Parasitol.* **1986**, *37*, 245−247.

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