Published in final edited form as:

Nat Prod Res. 2009 ; 23(7): 638–643. doi:10.1080/14786410802200479.

Antiproliferative Compounds of *Helmiopsis sphaerocarpa* from the Madagascar Rainforest†

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Abstract

Bioassay-directed separation of an ethanol extract of the leaves of *Helmiopsis sphaerocarpa* L.C. Barnett (Sterculiaceae) led to the isolation of the new compound 14 α ,15 α -epoxy-3 β -hydroxytaraxerane (**1**) and the four known compounds taraxerol (**2**), stigmast-5-en-3-ol (**3**), 5 α ,8 α -epidioxy-24(*S*)-methylcholesta-6,22-dien-3 β -ol (**4**), and 24 ξ -hydroperoxy-24-ethylcholesta-4,28(29)-dien-3-one (**5**). The structure of the new compound **1** was established on the basis of interpretation of its 1D and 2D NMR spectroscopic data. All the compounds were tested against A2780 human ovarian cancer cell lines, and compounds **4** and **5** showed mild antiproliferative activity with IC₅₀ values of 16 and 7 μ g/mL, respectively.

Keywords

Helmiopsis sphaerocarpa; Sterculiaceae; taraxerane; NMR; antiproliferative activity

1. Introduction

As part of our continuing investigation of Madagascar plants for compounds with antiproliferative activity, we found that an ethanol extract of the leaves of *Helmiopsis sphaerocarpa* L.C. Barnett showed moderate activity in the A2780 assay with an IC₅₀ value of 16 μ g/mL. The genus *Helmiopsis* has not been the subject of any significant chemical investigations, and the only published work is a study of the fatty acid from *H. richardii* (H. Bn) R. Cap. [2], and so this extract was selected for study. Bioassay-guided fractionation led to the isolation and characterization of five compounds from the extract. Taraxerol (**2**) [3], stigmast-5-en-3-ol (**3**), 5 α ,8 α -epidioxy-24(*S*)-methylcholesta-6,22-dien-3 β -ol (**4**) [4], and 24 ξ -hydroperoxy-24-ethylcholesta-4,28(29)-dien-3-one (**5**) [5] were known compounds, while compound **1** was new. Taraxerane-type triterpenoids have been isolated from several plant species, including *Bauhinia purpurea*, *Castanopsis lamontii*, and *Lithocarpus cornea* [6,7,8]. Here, we describe the structure elucidation of the new compound and the bioactivity of the isolates in the A2780 assay.

[†]Biodiversity Conservation and Drug Discovery in Madagascar, Part 33. For part 32, see reference [1]

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2. Results and discussion

The ethanol extract (MG 2490) was partitioned between hexane and 90% MeOH/H₂O, and then between dichloromethane and 70% MeOH/H₂O to give active hexane and dichloromethane fractions. The active hexane fraction was selected for bioassay-guided separation due to its similar activity to the dichloromethane fraction, but with larger amount. Separation of the hexane fraction over a silica gel open column furnished nine subfractions. Subfractions five and seven were purified by preparative silica gel TLC to yield 14 α ,15 α -epoxy-3 β -hydroxytaraxerane (**1**), taraxerol (**2**), stigmast-5-en-3-ol (**3**), 5 α ,8 α -epidioxy-24(*S*)-methylcholesta-6,22-dien-3 β -ol (**4**), and 24 ξ -hydroperoxy-24-ethylcholesta-4,28(29)-dien-3-one (**5**). The structures of compounds **2**, **4** and **5** were identified by comparison of their spectral data with previously published values, while the NMR and mass spectra of stigmast-5-en-3-ol (**3**) were identical to those of an authentic sample purchased from Aldrich. The optical rotation values of the known compounds are compatible with those reported in the literature.

14 α ,15 α -Epoxy-3 β -hydroxytaraxerane (**1**) was obtained as a colorless powder with a molecular formula of C₃₀H₅₀O₂ on the basis of its HRFABMS. Its ¹H NMR spectrum displayed signals corresponding to eight tertiary methyls at δ_H 1.08 (3H, s, H₃-26), 1.02 (6H, s, H₃-27 and H₃-28), 0.87 (3H, s, H₃-24), 0.84 (3H, s, H₃-25), 0.83 (3H, s, H₃-29), 0.77 (3H, s, H₃-30), and 0.66 (3H, s, H₃-23), and two oxygenated methines at δ_H 3.03 (1H, d, *J* = 6.9 Hz, H-15), 2.99 (1H, m, H-3). Its ¹³C NMR spectrum showed signals for 30 carbons, including one quaternary oxygenated carbon at δ_C 68.1 (C-14), two oxygenated methines at δ_C 76.5 (C-3), and 56.2 (C-15), and the other 27 carbons with chemical shifts from δ_C 10 to 52. On the basis of ¹H-¹H COSY, HMQC, and HMBC spectra, **1** was determined to be a taraxerane-type triterpenoid bearing one hydroxyl (C-3) and an epoxide (C-14/C-15) (Figure 1).

The ¹³C NMR spectrum of **1** was similar to that of taraxerol (**2**), except for the presence of signals corresponding to the carbons of an epoxide group at C-14 and C-15 the absence of signals for the carbons of a double bond (δ_C 158.1 and 117.0) [3]. Therefore, the planar structure of **1** was determined to be 14,15-epoxy-3-hydroxytaraxerane. In the 2D ROESY spectrum of **1**, H-15 correlated to H₃-26, and H₃-23 and H₃-24 exhibited correlations to H₃-25 and H-3, respectively (Figure 1). Thus, the structure was assigned as 14 α ,15 α -epoxy-3 β -hydroxytaraxerane.

It is noteworthy that three of the compounds isolated (**1**, **4**, and **5**) are oxidized triterpenoids. Although the known compounds **4** and **5** were first isolated from marine organisms [4,5], compound **4** has also been isolated from plants, for example from *Ganoderma lucidum* and *G. tsugae* [9]. Compound **5** has not previously been isolated from a terrestrial plant.

All the compounds were tested against the A2780 human ovarian cancer cell lines, and compounds **4** and **5** showed moderate activity with IC_{50} values of 16 and 7 $\mu\text{g/mL}$, respectively. Compounds **1-3** were not active against A2780 at 20 $\mu\text{g/mL}$, the highest concentration tested.

3. Experimental section

3.1 General experimental procedures

General experimental methods were as previously described [10].

3.2 Plant Material

The sample of *Helmiopsis sphaerocarpa* (Sterculiaceae) was collected in low dry forest in the Northern region of Madagascar, in the province of Antsiranana, in the limestone massif of Montagne des Français, at Ampitiliantsambo, 15 km North eastern of Andromanitra (12°23' 18"S 49°22'57"E, 230 m elevation) under the vernacular name Sely in June 2004. *Helmiopsis sphaerocarpa* is the only species among the nine currently known in this endemic genus of Madagascar to grow in the extreme north of the island. This species is morphologically unique

in the genus by bearing a spherical capsule instead of conical and by bearing glandular tissue both on the calyx lobes and on the petals (11). Duplicates of the voucher specimen (Rakotondrafara et al. 268) were deposited at the Missouri Botanical Garden, St. Louis, Missouri (MO), the Muséum National d'Histoire Naturelle, Paris (P), the Parc Botanique et Zoologique de Tsimbazaza, Madagascar (TAN), and the Centre National d'Application des Recherches Pharmaceutique, Madagascar (CNARP). The tree had a height of 14 m and trunk diameter at breast height of 11 cm.

3.3. Extraction

Field-dried leaves of *H. sphaerocarpa* (305 g) were ground in a hammer mill, then extracted by maceration in EtOH for 24 hours at room temperature and evaporated to give the crude extract MG2049 (11.4 g), of which 2.2 g was made available to Virginia Polytechnic Institute and State University.

3.4. Isolation

MG 2490 (1 g) was suspended in aqueous MeOH (MeOH-H₂O, 9:1, 50 mL) and extracted with hexane (3 × 50 mL portions). The aqueous layer was then diluted to 70% MeOH (v/v) with H₂O and extracted with CH₂Cl₂ (3 × 60 mL portions). Both the hexane and the CH₂Cl₂ extracts were evaporated *in vacuo* to leave 270 mg and 137 mg of residues (IC₅₀: 15 and 12 µg/mL, respectively). The aqueous MeOH extract was inactive. The hexane extract was selected due to its relatively greater quantity than the CH₂Cl₂ extract, and this was fractionated by flash chromatography over a Si gel column (2.3 × 4.5; 10g) using hexanes-EtOAc [(14:0 to 14:7, then 100% EtOAc, 50 mL × 9)] to furnish nine marginally active fractions (I, II, III, IV, V, VI, VII, VIII and IX). Fraction V yielded stigmast-4-en-3β-ol (**3**, 2 mg, *R_f* 0.5) and 5α, 8α-epidioxo-24(*S*)-methylcholesta-6,22-dien-3β-ol (**4**, 1.9 mg, *R_f* 0.25), while fraction VII afforded 14,15-α-epoxy-3-β-hydroxytaraxerane (**1**, 0.5 mg, *R_f* 0.65), taraxerol (**2**, 2.0 mg, *R_f* 0.6), and 24ξ-hydroperoxy-24-ethylcholesta-4,28(29)-dien-3-one (**5**, 2.3 mg, *R_f* 0.2) after separation by preparative Si gel TLC developed with CH₂Cl₂-MeOH (99:1).

3.4.1. 14,15-α-epoxy-3-β-hydroxytaraxerane (1)—Colorless powder; $[\alpha]_D^{23} +34^\circ$ (*c* 0.05, MeOH); UV (MeOH) $\lambda_{\max}$ 210nm; IR (film) $\nu_{\max}$ 3400, 2917, 2849, 1459, 1387, 1042 cm⁻¹; ¹H NMR (500 MHz, DMSO-*d*₆) 3.03 (1H, d, *J* = 6.9 Hz, H-15), 2.99 (1H, m, H-3), 1.08 (3H, s, H₃-26), 1.02 (6H, s, H₃-27 and H₃-28), 0.87 (3H, s, H₃-24), 0.84 (3H, s, H₃-25), 0.83 (3H, s, H₃-29), 0.77 (3H, s, H₃-30), 0.66 (3H, s, H₃-23); ¹³C NMR (125 MHz, DMSO-*d*₆) 76.5 (C-3), 68.1 (C-14), 56.2 (C-15), 55.1 (C-5), 49.2 (C-9), 45.5 (C-18), 40.5 (C-19), 39.5 (C-4), 38.7 (C-8), 38.3 (C-17), 37.5 (C-1), 37.4 (C-13), 36.9 (C-10), 36.0 (C-12), 34.1 (C-7), 33.4 (C-21), 32.3 (C-29), 31.2 (C-22), 30.6 (C-28), 29.2 (C-16), 28.6 (C-20), 27.9 (C-24), 26.8 (C-2), 24.1 (C-30), 23.9 (C-27), 23.3 (C-26), 22.0 (C-6), 17.3 (C-11), 15.9 (C-25), 15.8 (C-23); HRESIMS *m/z* 442.3831 [M]⁺ (calcd for C₃₀H₅₀O₂ 442.3811).

3.5. Antiproliferative assay

Measurements of antiproliferative activity were performed at Virginia Polytechnic Institute and State University against the A2780 ovarian cancer cell line as previously described [10]. The A2780 cell line is a drug - sensitive human ovarian cancer cell line. Paclitaxel was used as the positive control in the A2780 assay and it was active with an IC₅₀ value of 10 ng/mL.

Acknowledgments

This project was supported by the Fogarty International Center, the National Cancer Institute, the National Science Foundation, the National Heart, Lung and Blood Institute, the National Institute of Mental Health, the Office of Dietary Supplements, and the Office of the Director of NIH, under Cooperative Agreement U01 TW000313 with the International Cooperative Biodiversity Groups, and this support is gratefully acknowledged. We thank Dr. M. Callmender (Missouri Botanical Garden, Antananarivo) for assistance with the plant description, and Mr. B. Bebout

Mr. T. Glass (Virginia Polytechnic Institute and State University) for obtaining the mass spectra and for assistance with the NMR spectra, respectively. Field work essential for this project was conducted under a collaborative agreement between the Missouri Botanical Garden and the Parc Botanique et Zoologique de Tsimbazaza and a multilateral agreement between the ICBG partners, including the Centre National d'Applications des Recherches Pharmaceutiques. We gratefully acknowledge courtesies extended by the Government of Madagascar (Ministère des Eaux et Forêts).

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Figure 1.
Key 2D Correlations of **1**