Inhibition of *Mycobacterium tuberculosis* Growth by Saringosterol from *Lessonia nigrescens*

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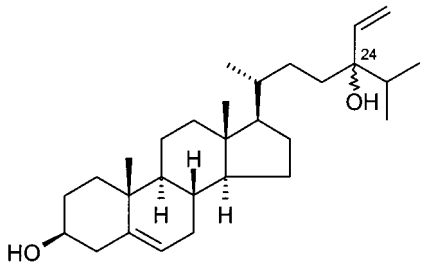
Assay-guided fractionation of an antitubercular extract obtained from *Lessonia nigrescens* yielded the phytosterol saringosterol as its active component. No appreciable toxicity against Vero cells was observed for this compound. Saringosterol was also synthesized by oxidation of fucosterol. The MIC values for antitubercular activity of saringosterol and its 24*S* and 24*R* epimers were determined as 0.25, 1, and 0.125 μg/mL.

The project "Bioactive Agents from Dryland Plants of Latin America", which is part of the International Cooperative Biodiversity Group (ICBG) program, routinely screens plant extracts from arid regions of Argentina, Chile, and Mexico for antitubercular activity.[1] The scope of this project has now been expanded to include organisms from coastal marine environments that serve as food sources for communities in coastal drylands. So far, we have reported on the isolation of an antitubercular diterpene alcohol[2] and the activities of several known and novel pentacyclic triterpenoids.[3−5] We now report on the antitubercular activity of the phytosterol saringosterol isolated from the Chilean brown algae *Lessonia nigrescens* Bory (Phaeophyta, Laminariales). In contrast to the antitubercular triterpenoids previously reported by us, saringosterol is distinguished by extremely low toxicity. Also, saringosterol had no appreciable effect on the growth of several mycobacteria, other than *M. tuberculosis*, or a panel of clinically relevant Gram-positive, Gram-negative, and anaerobic bacteria (data not shown). This is, to our knowledge, the first report on biological activity of this compound.

The crude dichloromethane−methanol extract obtained from *L. nigrescens* inhibited 99% of the growth of *M. tuberculosis* H_{37}Rv at a concentration of 100 μg/mL in the BACTEC 460 assay system.[6] The low toxicity of this extract against Vero cells[7] (IC_{50} > 128 μg/mL) suggested the presence of a selective antitubercular agent. Assay-guided fractionation led to the identification of saringosterol as the compound responsible for the antitubercular activity. This phytosterol had previously been isolated from the brown algae *Sargassum ringgoldianum*[8] and is known to be present in several other brown algae (Phaeophyta).[9] Its identity was established by comparison of NMR spectra, optical rotation, and melting point with published data.[8,10] Its MIC value vs *M. tuberculosis* was determined as 0.25 μg/mL, which is among the lowest values found for plant-derived natural products (compare to the tuberculosis drug rifampin, for which we also determined a MIC of 0.25 μg/mL in this assay).

Similar antitubercular activity has been reported for the phytosterol ergosterol endoperoxide.[11] Saringosterol showed no appreciable toxicity against mammalian cells in the Vero cell assay[7] (IC_{50} > 128 μg/mL). To allow further studies of this compound, we synthesized saringosterol from fucosterol, which is the major component of the sterol fraction of several brown algae including *L. nigrescens*. Fucosterol is readily available from giant kelp (*Macrocystis pyrifera*), which is used for the industrial production of alginates. We isolated fucosterol from the rotary filter mud, a waste product of this process.[12] Fucosterol itself showed no antitubercular activity. Oxidation with molecular oxygen in the presence of light and a sensitizer dye, such as Rose Bengal, and subsequent reduction of the intermediate hydroperoxide yielded a material that was found to be identical with the natural product by NMR, melting point, and optical rotation.[8,10] The semisynthetic material showed the same antitubercular activity against the H_{37}Rv strain of *M. tuberculosis* as the product isolated from *L. nigrescens*. Saringosterol isolated from *L. nigrescens* and semisynthetic material obtained from fucosterol are both 1:1 mixtures of 24*S* and 24*R* epimers. Separation of the isomers was achieved by normal-phase HPLC. The individual isomers were identified by comparison of their NMR data with published data.[10] In the BACTEC assay the 24*R* isomer was found to be 8 times more active against *M. tuberculosis* H_{37}Rv with a MIC of 0.125 μg/mL than the 24*S* isomer, which had a MIC of 1 μg/mL.

Saringosterol may be considered as an excellent lead structure for the development of urgently needed tuberculosis drugs due to its activity, specificity, and low toxicity.



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Experimental Section

General Experimental Procedures. NMR spectra were recorded in $CDCl_3$ on a Varian Unity 300 at 300 (^1H) and 75.4 MHz (^{13}C) with residual $CHCl_3$ ($\delta_H = 7.24$) and $CDCl_3$ ($\delta_C = 77.0$) as reference. Optical rotations were determined on a Jasco P1020 polarimeter. Melting points were taken on a Electrothermal apparatus. EI spectra were obtained with a Hewlett-Packard 5988A (70 eV) mass spectrometer. Visualization of compounds on silica gel TLC was carried out by spraying with a mixture of 0.5% anisaldehyde, 10% HOAc, and 5% H_2SO_4 in MeOH followed by heating. Si gel 60 (43−60 μm; Scientific Absorbents Inc., Atlanta, GA) was used for coumn chromatography (CC) and vacuum liquid chromatography (VLC).

Plant Material. *L. nigrescens* was collected by Luis Gonzalez in the district San Antonio of the Chilean Region V at a beach near Matanzas. A voucher specimen (no. 423) has been deposited at the herbarium of the Pontificia Universidad Católica de Chile.

Extraction and Isolation. The air-dried and ground plant material (1 kg) was extracted 3 times with MeOH−CH_2Cl_2 (1:1) at room temperature. The dried extract (10 g) was subjected to CC on Si gel using hexane−Me_2CO mixtures of increasing polarity. Antitubercular activity was concentrated in the fractions eluting with 10−20% Me_2CO. Saringosterol (10 mg) was isolated from these fractions by repeated CC.

Partial Synthesis of Saringosterol. Fucosterol used for the synthesis of saringosterol was isolated from the filter mud of industrial alginate production from giant kelp (*Macrocystis pyrifera*).[12] Fucosterol obtained by this process (1.25 g, 3.0 mmol) and Rose Bengal (125 mg) were dissolved in 750 mL of MeOH. While bubbling oxygen through the solution in a jacketed beaker at 5 °C, it was irradiated with a 500 W halogen lamp. After 26 h acetic acid (100 mL) and zinc dust (2 g) were added. The solvents were removed after 30 min of vigorous stirring, and the residue was subjected to VLC on Si gel with Me_2CO−CH_2Cl_2 mixtures of increasing polarity. From the fractions eluting with 3−4% Me_2CO 160 mg (13%) of starting material was recovered. Saringosterol (670 mg, 1.57 mmol, 52%) eluted with 6−8% Me_2CO.

Separation of Epimers. The HPLC separation of saringosterol epimers was carried out using a Varian 9002 pump equipped with a Varian Star 9040 refractive index detector. A normal-phase Si gel column (Alltech, Econosil 10 μm, 250 × 10 mm) was used with 4.5% Me_2CO in hexane at a flow rate of 5 mL/min, and the retention times of the epimers were 50.3 min (24*R*) and 53.0 min (24*S*).

Determination of Antitubercular Activity and Vero Cell Toxicity. Determination of antitubercular activity against *Mycobacterium tuberculosis* H_{37}Rv (ATCC 27294) in the BACTEC 460 assay system and assessment of cytotoxicity against Vero cells (ATCC CCL-81) in the CellTiter 96 aqueous nonradioactive cell proliferation assay were carried out as described earlier.[6,7]

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