A Cytotoxic Substance from Sangre de Grado

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Taspine has been isolated as a cytotoxic substance from Sangre de Grado, sap of Croton palanostigma (Euphorbiaceae), by bioassay guided fractionation. The cytotoxicity (IC₅₀) of taspine was found to be 0.39 µg/ml against KB cells and 0.17 µg/ml against V-79 cells.

Keywords Sangre de Grado; Croton palanostigma; Euphorbiaceae; taspine; alkaloid; cytotoxicity; V-79 cell; KB cell

Sangre de Grado, blood of gladness in Spanish, is red viscous sap produced by several Croton species (Euphorbiaceae) growing in the upper Amazon basin in Peru. The species from which it is usually obtained are Croton erythroxilus, C. lechleri, and C. palanostigma, also known as C. dracoides. This sap has been largely used by Peruvian natives for several medicinal purposes, including wound healing. Recently, Vaisberg and his collaborators reported the isolation of a potent wound healing substance from Sangre de Grado obtained from C. lechleri. Furthermore, Sangre de Grado has been widely used to treat cancer. As part of our continuing efforts to discover the antitumor potential of plant metabolites, Sangre de Grado obtained from C. palanostigma was found to be cytotoxic to V-79 cells (IC₅₀ = 3.7 µg/ml) in vitro. Bioassay directed purification guided by cytotoxicity against V-79 cells led to the isolation of taspine (I) as a cytotoxic substance.

Sangre de Grado obtained from C. palanostigma was freeze-dried, and chromatographed on a Diaion HP-20 column using stepwise elution [H₂O-MeOH (1:0−0:1) and 80% acetone]. The active fraction eluted with 100% MeOH was purified further by medium-pressure liquid chromatography (MPLC) on a silica gel column (CH₂Cl₂−MeOH, 13:7) to yield taspine (I).

Compound I was isolated as a white powder, mp >300°C. Its molecular ion peak (m/z 369) in electron impact-mass spectrum (EI-MS) and a positive Draganoff test suggested an alkaloid for I. The elemental analysis of I established the molecular formula as C₂₀H₁₉NO₆. The proton nuclear magnetic resonance (¹H-NMR) spectrum contained the signals due to two N-methyl [δ 2.44 (6H, s)], two methylene [δ 2.68 (2H, m), 3.45 (2H, m)], two methoxy [δ 4.12 (3H, s), 4.13 (3H, s)] and three aromatic protons [δ 7.22 (s), 7.35 (d, J = 8.8 Hz), 8.09 (d, J = 8.8 Hz)]. These data suggested I to be taspine, and the structure was identified in comparison with the published data. The carbon-13 nuclear magnetic resonance (¹³C-NMR) data for I, which had not previously been reported, were determined by ¹H−¹³C shift correlation via long-range coupling (COLOC) experiments (Table I).

The cytotoxic activities of taspine are listed in Table II. Taspine is the alkaloid previously isolated from several families, including Berberidaceae, Euphorbiaceae, and Magnoliaceae. The biological activities of Sangre de Grado and/or taspine have been reported with respect to an anti-inflammatory, a reverse transcriptase inhibitor, and wound healing activities. In this study, taspine was revealed to be the cytotoxic substance of Sangre de Grado and showed strong cytotoxicity as a plant metabolite.

Experimental
Melting point (uncorrected) was determined on a Yanagimoto micro melting point apparatus, infrared (IR) spectrum on a Perkin-Elmer 1710 FTIR spectrometer, ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) were recorded on a Bruker AM-400 spectrometer in CDCl₃−CD₃OD (3:1) with tetramethylsilane (TMS) as an internal standard. Mass spectrum (MS) was obtained on a Hitachi M-80 spectrometer. For column chromatography, Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) as highly porous synthetic resin was used. MPLC was carried out with a CIG column system (Kusano Scientific Co., Ltd.) on a 10 µm silica gel. Plant Material Sangre de Grado was obtained in Iquitos, Peru, in August 1987. The material, which was obtained from C. palanostigma, was identified by Dr. F. Ayala Flores (Peru Amazon University). Isolation The freeze-dried sap (70 g) was chromatographed on a Diaion HP-20 with H₂O, 10% MeOH, 20% MeOH, 40% MeOH, MeOH, and 80% acetone. The MeOH eluted material was purified by silica gel MPLC

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δ in CDCl₃−CD₃OD (3:1)

Table II. Cytotoxic Activity of Taspine (I)

<table>
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<tr>
<td>V-79 cells</td>
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with CH₂Cl₂-MeOH (13:7) to afford taspine (700 mg).

Taspine (I): A white powder, mp > 300°C. EI-MS (rel. int.) m/z: 369 (7, M⁺), 58 (100). IR (CHCl₃): 3010, 2943, 2848, 2827, 2786, 1738, 1600, 1472, 1439, 1295, 1135, 1090 cm⁻¹. ¹H-NMR: 6: 2.44 (6H, s, N-CH₃ x 2), 2.68 (2H, m, H₂-α), 3.45 (2H, m, H₂-β), 4.12 (3H, s, 6-O-CH₃), 4.13 (3H, s, 1-O-CH₃), 7.22 (1H, s, H-2), 7.35 (1H, d, J = 8.8 Hz, H-7), 8.09 (1H, d, J = 8.8 Hz, H-8). Anal. Calcd for C₁₆H₁₄NO₄: C, 65.03; H, 5.18; N, 3.79. Found: C, 64.75; H, 5.12; N, 3.72.

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References and Notes