(+)-*epi*-α-Bisbolol Is the Wound-Healing Principle of *Peperomia galioides*: Investigation of the in Vivo Wound-Healing Activity of Related Terpenoids

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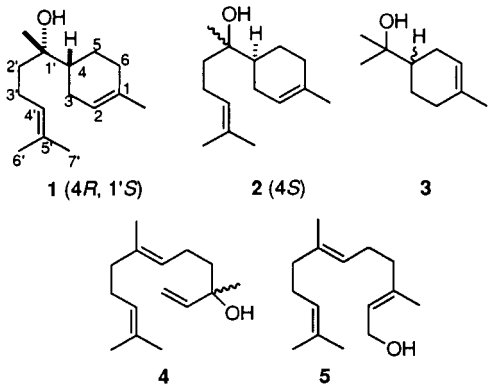
Bioassay-guided investigation of the ethanol extract of *Peperomia galioides* using a tensile strength method in mice as a monitor led to the isolation of *epi*-α-bisabolol (**1**) (ED_{50} 155 μg/mL). An in vivo healing study of selected commercially available monoterpenoids yielded two bioactive compounds, α-bisabolol (**2**) and α-terpineol (**3**) (ED_{50} of 228 and 240 mg/g mouse, respectively).

Impaired wound-healing is a significant source of morbidity and may result in severe health-related complications, such as infections and tissue necrosis.[1] Impaired wound-healing is associated with several conditions such as diabetes, immunosuppression, obesity, and malnutrition. The scientific study of wound-healing (cicatrizant) plants, which can promote or aid in the normal wound-healing process, could help patients whose wounds do not scar easily. Notwithstanding these medical benefits, an in-depth study of wound-healing agents may also expose a potential link between acute injury and the treatment of proliferative disorders.[2] Our initial foray in the field of natural cicatrizants was the study of the tree sap from *Croton lechleri* L. (Euphorbiaceae) ("Sangre de Grado"), known in folk medicine for its healing action. This investigation resulted in the isolation of taspine, the wound-healing principle in the sap.[3] This alkaloid accelerates the healing process, presumably, by increasing the migration of fibroblasts to the wounded area during the early stages of cicatrization.[4] Our group's focus on cicatrizant agents from natural sources is part of an interdisciplinary, systematic, and targeted study of traditional Peruvian medicinal plants.[5] In the past decade, the number of publications related to *C. lechleri* and other natural wound-healing agents has increased substantially,[6] including our recent survey of Peruvian plant species with potential cicatrizant action.[7] One such plant, *Peperomia galioides* H.B.K. (Piperaceae) ("Congona"), is a succulent herb, used in traditional medicine as a compress—made from the crushed plant except the roots—that is applied on cuts and wounds to accelerate healing. The juice is swallowed to treat gastric ulcers. We found that the ethanol extract of *P. galioide*s exhibited significant wound-healing activity in vivo, without any increase in the proliferation of 3T3 mouse fibroblasts and with no detectable mutagenicity.[8] Cavé and co-workers have published the only previous phytochemical investigation of *P. galioides.* They isolated, from the petroleum ether extract, a prenylated quinone with in vitro antiparasitic activity.[9] We report now the isolation of (+)-*epi*-α-bisabolol, **1**, responsible for the in vivo cicatrizant activity of *P. galioides*, and also provide a preliminary account of our investigation on the wound-healing activity of terpenols **2**−**5**.



The ethanol extract of *P. galioides* was partitioned between water and dichloromethane, resulting in an enhanced activity of the latter fraction. Further partitioning (90% aqueous methanol/hexane) concentrated the bioactivity in the hexane layer. Chromatographic purification (radial chromatography and HPLC) of the hexane extract led to the isolation of (+)-*epi*-α-bisabolol (**1**). The optical rotation and NMR spectra of **1** were identical to those reported in the literature,[10−13] including the characteristic C-1′ methyl singlet (δ 1.14 ppm in $CDCl_3$). Because the (−)-enantiomer is known as (−)-anymol,[11] some authors[12] prefer to use the name (+)-anymol when referring to **1**. Other components of *P. galioides*, isolated during the bioassay-guided fractionation procedure, included the methyl ester of grifolic acid,[14] grifolin,[14] and viridiflorol.[15] The cicatrizant activity (ED_{50}) of **1**, determined using a tensile strength method,[8] and its cytotoxicity against 3T3 mouse fibroblasts (GI_{50})[3] are shown in Table 1.

With an interest in seeking readily available sources of cicatrizant agents, as well as to begin to understand the structural features needed for their wound-healing activity, we examined the in vivo healing action of selected and commercially available terpenols whose carbon skeletons

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Table 1. Wound Healing Activity and Cytotoxicity for Compounds **1**−**3** Expressed as ED_{50}[a] and GI_{50}, Respectively

	(+)-*epi*-α-bisabolol (**1**)	α-bisabolol racemate (**2**)	α-terpineol racemate (**3**)
ED_{50} (μg/g mouse)	155	228	240
GI_{50} value against 3T3 cells (μg/mL)	8.0	84	17

[a] ED_{50} values were calculated from the regression equation obtained from a dose−response analysis. Statistical analysis using the Student's *t*-test showed that the difference between the control group and the treated group of mice was significant ($p < 0.05$). The control experiments utilized mice who received only the solvent in which the compound was diluted.

bear some resemblance to **1**, namely, α-bisabolol (**2**), α-terpineol (**3**), *trans*-nerolidol (**4**), and *trans*,*trans*-farnesol (**5**). α-Bisabolol (**2**), the epimer of **1**, is a common plant secondary metabolite. Its (−)-enantiomer is the main constituent of chamomile (*Matricaria chamomilla* L.) and has been shown to shorten the healing time in cutaneous burns of guinea pigs exposed to UV light.[16] In terms of its structure, **3** displays a skeletal arrangement similar to **1**, minus the isoprene chain, while **4** is an acyclic isomer of **1** bearing a tertiary alcohol, albeit on a different location. On the other hand, **5**, another acyclic isomer of **2**, features a primary alcohol instead of the tertiary hydroxyl group.

Our results indicate that whereas both **2** and **3** showed significant in vivo cicatrizant activity (Table 1), the acyclic isomers **4** and **5** were inactive; that is, their wound breaking strength data were lower or similar to the control experiments. For comparison, the ED_{50} of taspine was 15 μg/g mouse, but its cytotoxicity was much greater than **1**−**3**.[17] Thus, compared with taspine, terpenols **1**−**3** are less potent, but also much less cytotoxic, and more accessible in terms of their chemical synthesis. For example, whereas the total synthesis of taspine has only recently been achieved,[18] compounds **2** and **3** are commercially available, and **1** has been prepared using various synthetic routes.[10,19] One might speculate that the 1-methylcyclohexene moiety and the tertiary hydroxyl group on C-1′ are important structural fixtures for the wound-healing enhancement observed in **1**−**3**, while the isoprene side chain and the stereochemistry on C-4 may be less relevant for wound-healing activity.[20] Migration of fibroblasts is an important parameter in the cicatrization process (vide supra), since disturbance in the arrangement of a tissue, by wounding, normally initiates migration of the surrounding cells to the wounded area. We tested the effect of **1** on cell migration by inflicting a "wound" on a cell monolayer, using our published procedure,[3] but found that this compound did not have a significant effect on increasing cell migration.

Experimental Section

General Experimental Procedures. Commercially available samples of α-bisabolol (**2**) (Fluka 14462), *trans*-nerolidol (Aldrich 33,525-8) (**3**), α-terpineol (Aldrich 43,262-8) (**4**), and *trans*,*trans*-farnesol (Aldrich 27,754-1) (**5**) were purchased and used without further purification. Optical rotations were obtained on a Perkin-Elmer model 241 polarimeter. ^{1}H and ^{13}C NMR data were measured in $CDCl_3$ at 300 and 75 MHz, respectively (Bruker AC 300 FT-NMR), referenced against internal tetramethylsilane at δ 0.0 ppm. *J* values are given in Hz. Low-resolution EIMS were recorded on a HP 5890/5970 GCMS. HRMS data were obtained at the Midwest Center for Mass Spectrometry, University of Nebraska−Lincoln. Analytical TLC was performed using Macherey-Nagel Polygram Sil G/UV_{254} precoated plates. Column chromatography utilized Si gel, 63−200 μm (Scientific Adsorbents, Inc.). Radial chromatography was performed on 1 or 2 mm Si gel coated circular glass plates using a Chromatotron apparatus. High-performance liquid chromatography was carried out using a Rainin gradient HPLC system (Dynamax SD-200 pumps) with UV-M multiwavelength detector.

Plant Material. *Peperomia galioides* H.B.K. (Piperaceae) was collected between the months of June and August (1993−1995), in sheltered areas of the ravine of Vicus, in the Department of Ancash, Province of Carhuaz, District of Marcará, Peru, at 3900 m altitude. I.D.F. conducted the botanical identification. A voucher specimen (IFV320) has been deposited at the Museo de Historia Natural Javier Prado of the Universidad Nacional Mayor de San Marcos in Lima, Perú.

Extraction and Isolation. Fresh *P. galioides* (whole plant, 5.6 kg) was crushed and macerated with ethanol at room temperature, yielding 72 g of crude extract after evaporation of the solvent (tensile strength[8] activity: 36%, using 16 mg per mouse). The extract was partitioned between H_2O and CH_2Cl_2, and the dichloromethane layer (20.4 g) was concentrated and further partitioned between hexane (6.2 g) and aqueous methanol 90% (12.6 g). An aliquot of the hexane fraction (1.90 g) was subjected to repeated purification using column and radial chromatography (eluent CH_2Cl_2). The following compounds were isolated and identified during the purification process: grifolin[14] (140 mg), viridiflorol[15] (5 mg), and the methyl ester of grifolic acid[14] (38 mg). The subfraction with R_f between 0.4 and 0.5 (hexane/ethyl acetate, 85:15) was further chromatographed, using preparative HPLC (Si gel, hexane/*i*-PrOH, 95:5), to afford (+)-*epi*-α-bisabolol **2** (18 mg) (tensile strength[8] activity: 62% using 4 mg per mouse).

(+)-*epi*-α-Bisabolol (1): colorless oil, $[\alpha]^{23}_D$ +43.95° (*c* 0.38, $CHCl_3$) (lit.[10] $[\alpha]^{23}_D$ +45.3°); HRMS *m*/*z* 204.18737 ($M^+ - H_2O$, deviation: −2.12 ppm); and exhibited spectral data (^{1}H NMR, ^{13}C NMR; EIMS) values comparable to literature data.[10−13,19]

Biological Assays. The methods used to determine the in vivo wound-healing activity[3,8] and cytotoxicity[5b,c] have been reported.

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