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Research Article

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Biological activity of 1-heneicosanol isolated from *Senecio coluhuapiensis*, an endemic species from Patagonia, Argentina

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Abstract The genus *Senecio* from Patagonia, Argentina, contains several species, including *Senecio coluhuapiensis.* In the present study, we isolated 1-heneicosanol from this angiosperm. The structure of 1-heneicosanol was established on the basis of spectroscopic analysis and bibliographical data, and its microbiological activity evaluated. 1-heneicosanol presented activity against *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853) as well as against *Candida albicans* (NIM 982879) and *C. krusei* (ATCC 6258), at a concentration of 250 µg/ml for all species. The most active concentration of pure compound for reducing the germinative tube of the phytopathogenic fungus *Botrytis cinerea* was 80 µg/ml.

Keywords *Senecio coluhuapiensis*, fatty alcohols, antimicrobial activity

Introduction

The genus *Senecio* (Asteraceae) is one of the richest in species among angiosperms. It includes almost 3000 species dispersed all over the world, except Antarctica and the Amazonian region. In Argentina, there are more than 270 species distributed in the Andes and Patagonia.

These species present a varied morphological and chemical diversity. Different kinds of compounds, such as pyrrolizidine alkaloids [1], furanoeremophilanes and essential oils [2], have been studied in many species of this genus. *Senecio coluhuapiensis* Spegazzini, a species endemic to the Petrified Forest of Sarmiento, Chubut, Argentina, is associated with the Salamanca formation, characterized by a barely developed ground and an extremely acid pH [3]. The knowledge of the biological and chemical aspects of this species may allow determining its state of conservation. This knowledge may also contribute to designing conservation programs for its survival and reproduction in conditions of poor and barren grounds. The aim of the present study was to find whether 1-heneicosanol [4] has biological activity. To this end, this compound was isolated from *Senecio coluhuapiensis* and its chemical structure established on the basis of spectral analysis and bibliographical data. This compound was analyzed for its antimicrobial activity against bacteria and yeast, and for its antifungal activity against *Botrytis*

cinerea, a phytopathogenic fungus that causes many losses in grape crops. The sesquiterpene botrydial and other related metabolites produced by this fungus are responsible for its phytotoxic activity, which produces necrotic lesions in the host plant [5]. An important strategy for the control of *B. cinerea* is based on the inhibition of the biosynthesis of these metabolites [6].

Materials and Methods

Collection of Plants Materials

Aerial parts of *Senecio coluhuapiensis* were collected in Sarmiento (province of Chubut, Argentina) in May 2009. A voucher specimen was authenticated and deposited in the Patagonia Regional Herbarium (HRP 7165) of Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), Comodoro Rivadavia, Argentina.

Extraction and Isolation

Aerial parts of *S. coluhuapiensis* were dried, pulverized (93.32g) and extracted exhaustively with ethanol at 95° at room temperature. The solvent was evaporated under reduced pressure to afford 6.3 g of crude extract. The whole extract was fractionated by column chromatography (CC): Silica gel 60 (SiO_2; (230-400 mesh); Merck) and eluted with mixtures of hexane/AcOEt of increasing polarity [9]. The fractions were analyzed by thin layer chromatography (Silica gel 60 $F_{254,}$ Merck, 0.2 mm thickness) eluted with hexane/AcOEt 90:10 as mobile phase and then revealed by spraying a 25 % H_2SO_4 solution followed by heating. Pure compound (0.17 g) was obtained from the hexane/AcOEt 80:20 mixture.

General Experimental Procedures

All 1D and 2D NMR spectra (COSY, HSQC and HMBC) were recorded on a spectrometer of Magnetic Resonance Nuclear Bruker Advance II 500 (1H to 500.13 MHz; 13C to 125.77 MHz). The processing software was Topspin 2.1 (Bruker) in $CDCl_3$ solution δ in ppm relative to Me_4Si as internal standard, J in Hz.

IR spectra were measured on a Nicolet Magna 550 FT-IR. Solid spectra: dispersion 1% in KBr. If the sample was not large enough film by evaporation of solution in CH_2Cl_2. MS spectra were measured on a Shimadzu QP-5050 A mass spectrometer. Direct introduction and ionization by electronic impact to 70 eV.

1-heneicosanol (**1**) Yield: 170.2 mg. Crystalline white solid. M.p. 68 º (hexane/AcOEt). FT-IR: 3442, 2919, 2850, 1735, 1466, 724 cm^{-1}. The ^{1}H-NMR spectrum displayed the typical signals (Table 1) 0.88 (3H, t, J=7 Hz, CH_3), 1.25 (2H, m, $(CH_2)_n$), 1.57 (2H, t, J=6.5 Hz, CH_2), 3.65 (2H, t, J=6.5 Hz, CH_2-OH). In the ^{13}C RMN spectrum showed different C-atom (Table 1) 14.1 (CH_3), 22.7 (CH_2), 25.7 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 31.9 (CH_2), 32.8 (CH_2), 63.1 (CH_2). EI-MS (70 eV): 312(3, M^+), 227(5), 125(9), 111(18), 97(35), 83(48), 69 (52), 57(77), 43 (100).

Botrytis cinerea. Isolation and Culture Conditions

B. cinerea was isolated from infected grapes (province of Mendoza, Argentina). The test consisted in placing 2 µl of a suspension of gray-mold spores on slides with 1 X 10^6 spores/ml and 8 µl of a solution in dimethyl sulfoxide (DMSO) of the pure compound isolated from *Senecio coluhuapiensis*, to obtain the concentrations studied (5, 10, 20, 40, 80, 120, 160, 180 µg/ml). The controls were made equally single with 8 µl of distilled water. Each treatment was repeated twice. Each concentration was placed in plastic Petri dishes, with a dampened paper, to avoid the dehydration of the sample. The incubation was carried out for 16 hours in the dark at room temperature. The test stopped with the addition of Güegüen stain. The germination capacity was evaluated by means of observing the slides under an optical microscope, to determine the number of germinated propagules, considering germinated those whose germinative tube had a length equal to or greater than the length of the conidium. The length of the germinative tube was also measured. The collected data were subjected to statistical analysis of average variance and separated by means of Tukey test and LSD. The statistical program SPSS 9.0 was used.

Antimicrobial and Antifungal Activity

The compound was assayed against eight microorganisms including Gram-positive and Gram-negative bacteria and yeast: *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25299), *Candida albicans* (NIM 982879/982891), *Candida parapsilopsis* (ATCC 22019), and *Candida krusei* (ATCC 6258), donated by ANLIS Malbrán Institute, Buenos Aires, Argentina. The antimicrobial activity was determined in solid phase by the Agar Dilution Method [10], using sterile nutritive agar for bacteria and 4% glucose-

Sabouraud as culture medium for yeasts. An aliquot from a 2500 µg/ml stock solution of 1-heneicosanol in DMSO was inoculated into the liquid warm medium to obtain different final concentrations (250, 100 and 50 µg/ml), then stirred for 1 min in a vortex at 3000 rpm and allowed to solidify. An inoculum of 106 CFU/ml was applied on the medium surface, and then incubated for 24 h at 37 ºC for bacteria and for 48 h at 28 ºC for yeasts. The minimal inhibitory concentration (MIC) endpoint was determined visually by recording the lowest concentration of the compound that prevented the appearance of visible growth.

Results and Discussion

1-heneicosanol was isolated by column chromatography (CC). Spectroscopic techniques such as 1D- and 2D-NMR (Table 1) as well as EI-MS were used for structure elucidation. The EI-MS analysis showed an ion peak at *m/z* 312 (M^+), concordant with the molecular formulae $C_{21}H_{44}O$ (Fig. 1).

The 1D- and 2D-NMR spectra allowed inferring the presence of one Me and twenty CH_2 groups. The ^{1}H-NMR spectra (Table 1) displayed the typical signals assigned to δ (H) 0.88 (*t*, J= 7, Me-(C21)) and δ (H) 3.65 (*t*, J= 6.5, H-(C1)). In the HMBC spectrum (Table 1), correlations were observed between the H-atom resonance of Me (21) (δ(H) 0.88) and that of C(20); the resonance of H-C(1) (δ(H) 3.65) and that of C(2), and the resonance of H-C(2) (δ(H) 1.57) and that of C(4). The presence of the OH group was deduced from the FT-IR absorption at 3492 cm^{-1} and by the signal at 63.13 ppm in the ^{13}C-NMR spectrum. The location of the OH group at C(1) was established by correlation cross-peaks between H-C(1) and H-C(2) in the ^{1}H,^{1}H-COSY spectrum.

Figure 1: 1-heneicosanol

Table 1: ^{1}H-, ^{13}C-, HMBC-, HSQC and COSY- NMR. Data for 1-heneicosanol [a] ($CDCl_3$)

Carbon signal	Chemical group	HSQC		HMBC	COSY
		^{13}C-NMR	^{1}H-NMR		
1	CH_2	63.13	3.65 t (6,5Hz)	32.81	H-2
2	CH_2	32.81	1.57 t (6,5Hz)	29.67	H-3, H-4, H-5, H-6, H-7
3	CH_2	31.93	1.25 m		H-3, H-4, H-5, H-6, H-7
4	CH_2	29.67	1.4 m		H-3, H-4, H-5, H-6, H-7
5	CH_2	29.61	1.4 m		H-3, H-4, H-5, H-6, H-7
6	CH_2	29.44	1.4 m		H-3, H-4, H-5, H-6, H-7
7	CH_2	25.74	1.4 m		H-3, H-4, H-5, H-6, H-7
8	CH_2	22.70	1.32 m		H-3, H-4, H-5, H-6, H-7
9	CH_3	14.13	0.88 t (7Hz)	22.70	H-3, H-4, H-5, H-6, H-7

[a] ^{1}H and ^{13}C-NMR spectra were recorded at 500 and 127 MHz, respectively.

Phytopathogenic Activity against *Botrytis cinerea*

1-heneicosanol showed antifungal activity against *B. cinerea*. The effect of different concentrations of 1-heneicosanol in the mean length of the germinative tubes obtained from conidia of *B. cinerea* is shown in Fig. 2. The most active concentration of compound 1 for reducing the germinative tube in *B. cinerea* was 80 µg/ml (Table 2). Intermediate concentrations (20 and 40 µg/ml) showed lower activity. The compound was not active at concentrations from 5 to 10 µg/ml.

Antimicrobial and Antifungal Activity

1-heneicosanol exhibited antifungal activity against *Candida albicans* and *C. krusei*, with MIC values of 250 µg/ml for both yeast species. 1-henicosanol was tested against two Gram-negative bacteria (*Escherichia coli, Pseudomonas*

aeruginosa) and one Gram-positive bacterium (*Staphylococcus aureus*). The compound was active against *S. aureus* and *P. aeruginosa*, showing MIC values of 250 µg/ml, in both cases (Table 3).

Table 2: Growth inhibition of the length of the germinative tube of *B. cinerea* for different concentrations of 1-heneicosanol[a]

1-heneicosanol (µg/ml)	Mean ± standard error
0	224.2 ± 9.7[a]
5	224.1 ± 5.5[a]
10	231.2 ± 7.0[a]
20	197.1 ± 8.1[b]
40	110.8 ± 3.1[c]
80	12.6 ± 1.0[d]
120	0.0 ± 0.0[d]
160	0.0 ± 0.0[d]
180	0.0 ± 0.0[d]
200	0.0 ± 0.0[d]

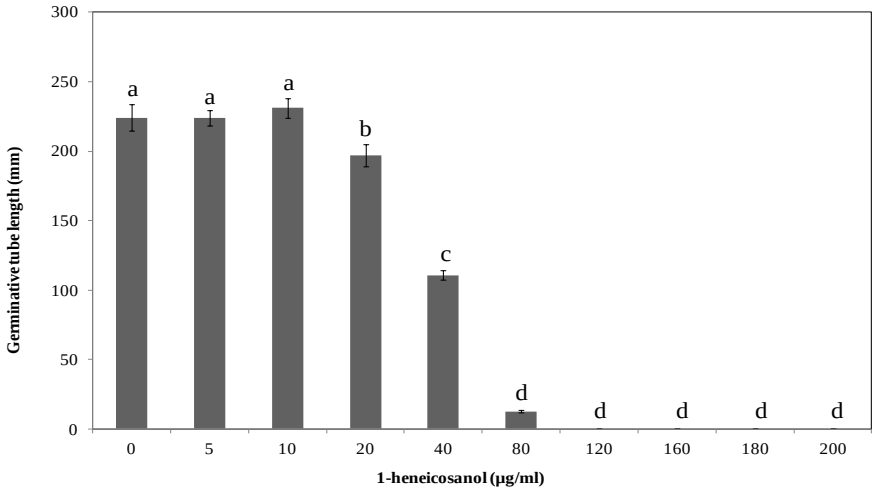

Figure 2: Growth inhibition of the length of the germinative tube of B. cinerea for different concentrations of 1-heneicosanol

Table 3: Antimicrobial and antifungal activity of 1-heneicosanol from *S. coluhuapiensis* expressed as minimal inhibitory concentration (MIC)

Microorganism	MIC (µg/ml)
Candida albicans (NIM 982879)	250
Candida albicans (NIM 982891)	250
Candida krusei (ATCC 6258)	250
Candida parapsilopsis (ATCC 22019)	(-)[a]
Staphylococcus aureus (ATCC 29213)	250
Escherichia coli (ATCC 25299)	(-)[a]
Pseudomonas aeruginosa (ATCC 27853)	250

[a)] No antimicrobial activity observed at the concentrations tested.

Conclusions

In conclusion, this study demonstrates the *in vitro* activity of 1-heneicosanol isolated from *Senecio coluhuapiensis* against the gray mold disease. However, in order to evaluate their potential as preventive treatments, further studies

and *in vivo* testing are required to determine the effect of this compound on spore germination. In addition, 1-heneicosanol was also found to be active against Candida sp and *S. aureus* and *P. aeruginosa* [7-8]. The presence of a polar group at both ends of the non-polar chain may prevent the binding of the molecule to the membrane of the cells. Thus, further studies about the applications of this kind of compounds should be carried out [9-12].

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List of Abbreviations: HRP (Herbario Regional Patagónico); ASTM (American Society for Testing and Materials); ATCC (American Type Culture Collection); NIM (Número Instituto Malbrán); MIC (Minimal Inhibition Concentration); ANLIS (Administración Nacional de Laboratorios e Institutos de Salud).

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