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Artigo

Sesquiterpenoids of *Senecio bonariensis* Hook. & Arn., Asteraceae

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RESUMO: "Sesquiterpenóides de *Senecio bonariensis* Hook. & Arn., Asteraceae". Das folhas de *Senecio bonariensis* Hook. & Arn. foram isolados três sesquiterpenóides. β-Cariofileno (1), óxido de β-cariofileno (2) e germacreno D (3) foram identificados por CG-IE-EM, IV, ^{1}H and ^{13}C-RMN. A importância ecológica e quimiotaxionômica para o gênero *Senecio* é discutida, assim como a correlação biossintética entre as substâncias isoladas. As substâncias **1** e **2** tiveram sua atividade antimicrobiana avaliada pelo método de microdiluição em caldo contra cepas bacterianas e fúngicas. Ambas não inibiram o crescimento microbiano nas concentrações testadas.

Unitermos: *Senecio bonariensis,* β-cariofileno, óxido de β-cariofileno, germacreno D, quimiotaxonomia, atividade antimicrobiana.

ABSTRACT: From the leaves of *Senecio bonariensis* Hook. & Arn. three sesquiterpenoids were isolated. β-caryophyllene (**1**), β-caryophyllene oxide (**2**) and germacrene D (**3**) were characterized by GC-EI-MS, IR, ^{1}H and ^{13}C-NMR data. Their ecological and chemotaxonomical significance for the genus *Senecio* are discussed, as well as the biosynthetic correlation between the isolated substances. The antimicrobial activity of substances **1** and **2** were evaluated by the microdilution method against bacterial and fungal strains. Both compounds did not inhibit the microorganism growth at the tested concentrations.

Keywords: *Senecio bonariensis,* β-caryophyllene, β-caryophyllene oxide, germacrene D, chemotaxonom y, antimicrobial activity.

INTRODUCTION

Species belonging to the family Asteraceae are an important source of terpenes with biological activity (Reina et al., 2001). The genus *Senecio* (Tribus Senecioneae, Asteraceae) has more than 2000 species. In Brazil, 85 species are been described and form these, 33 occur in southern of the country and 25 were identified in the state of Rio Grande do Sul. *Senecio bonariensis* Hook. & Arn. (Asteraceae), popularly known as "margarida-do-banhado-de-buenos aires" is native from South America and can be found in South Brazil, blooming from October to December (Cabrera & Klein, 1975; Matzenbacher, 1998). This aquatic plant is known for containing furanoeremophilanes (Pomílio & Jares, 1997; Tettamanzi et al. 1992), steroids (Jares et al., 1990; Tettamanzi et al., 1992) and the pyrrolizidine alkaloids senecionine and platyphylline (Paiva et al., 2004; Silva et al., 2006; Tettamanzi et al., 1992) with toxic properties. This species is used in traditional medicine for the treatment of skin, respiratory and osteoarticular diseases (Bolzan et al., 2007).

In this article we describe the isolation and identification of three compounds from the CH_2Cl_2 extract of the aerial parts of *S. bonariensis* and their antimicrobial evaluation.

MATERIALS AND METHODS

Plant material

Leaves of *Senecio bonariensis* Hook. & Arn., Asteraceae, were collected in Eldorado do Sul-RS, Brazil, in April 2004 and identified by Prof. Dr. Nelson

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Ivo Matzenbacher. Voucher specimen Nº SMDB 9519 is preserved in the Herbarium of the Departamento de Botânica, Universidade Federal de Santa Maria, Santa Maria-RS, Brazil.

Extraction and purification

Fresh leaves of *S. bonariensis* (2.3 kg) were extracted by maceration with CH_2Cl_2, two times. The combined CH_2Cl_2 extract was evaporated under vacuum to yield a viscous residue (13.5 g). The crude extract was fractionated by flash chromatography over silica-gel (Merck, 230-400 mesh), using CH_2Cl_2, and CH_2Cl_2-EtOH mixtures of increasing polarity (stepwise, 100:0 to 80:20), to yield fifteen fractions. The fraction 1 of column 1 (1 g) was chromatographed over $AgNO_3$-impregnated silica-gel (Stahl, 1969) eluting with *n*-hexane-acetone (99:1), to yield thirteen new fractions. Fraction 10 of column 2 (253 mg) was chromatographed on $AgNO_3$-impregnated silica-gel, eluting with hexane-ethyl ether (95:5) to afford 104 mg of **1** and 16 mg of **2**. Fraction 11 of column 2 (83.5 mg) was also chromatographed on $AgNO_3$-impregnated silica-gel, eluting with *n*-hexane:ethyl-ether (98:2) to afford 5 mg of **3.** The compounds obtained were analyzed by GC-EI-MS, IR, ^{1}H and ^{13}C NMR. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC.

Spectroscopic analysis

GC-MS were performed using a Hewlett Packard 6890 GC system with a fused capillary column (30 m x 0.25 mm x 0.25 µm, HP-5MS, 5%-phenyl - 95%-methylsiloxane, HP) coupled to Hewlett Packard 5973 mass detector. EI-MS TIC: 70 eV; Operating conditions: split injection, split ratio approx. 1:100; temperature programme, 40-260 ºC; ramp rate, 4 ºC/min; carrier gas He: flow rate, 1 mL/min.; injector temperature, 220 ºC; detector temperature, 220 ºC. Data bank NIST, 1998. IR spectra were taken on a Nicolet Magna 550, coupled with HATR for liquid samples and DRIFTS for the solid one. NMR spectra were recorded on a Bruker DPX 300 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C, in $CDCl_3$ using TMS as internal standard.

β-Caryophyllene (**1**): pale yelow oil; R_f = 0.56 ($AgNO_3$-impregnated silica-gel F_{254}, *n*-hexane:ethyl acetate (97:3), three developments, visualization by spraying anisaldehyde-H_2SO_4 reagent); EI-MS *m/z* 204 $[M]^+$ (4), 189 (10), 175 (5), 161 (18), 147 (15), 133 (50), 120 (24), 105 (39), 93 (65), 91 (66), 79 (60), 69 (63), 55 (35), 41 (100); calcd for $C_{15}H_{24}$ 204 g; ν_{max} 2930, 1450, 885 cm^{-1}; ^{1}H NMR: δ 5.29 (*m*, 1H; H-5), 4.82, 4.94 (2 *s*, 2H; H-15_a and H-15_b), 2.33 (*q, J* = 9.3 Hz, 1H; H-9), 2.00 - 2.20 (*m*, 4H; H-6 and H-7), 1.91, 1.99 (2 *m,* 2H; H-3_a and H-3_b), 1.69 (*m*, 1H; H-1), 1.52, 1.67 (*m,* 2H; H-10_a and H-10_b), 1.61 (*s*, 3H; H-14), 1.26 (*m*, 2H; H-2), 1.00 (*s*, 3H; H-12), 0.97 (*s*, 3H; H-13); ^{13}CNMR: δ 154.7 (C-8), 135.5 (C-4), 124.4 (C-5), 111.6 (C-15), 53.7 (C-1), 48.5 (C-9), 40.4 (C-10), 40.0 (C-3), 34.8 (C-7), 33.0 (C-11), 30.1 (C-13), 29.4 (C-6), 28.4 (C-2), 22.6 (C-12), 16.3 (C-14).

Caryophyllene oxide (**2**): colorless needles; R_f = 0.67 ($AgNO_3$-impregnated silica-gel F_{254}, *n*-hexane:ethyl acetate (97:3), three developments, visualization by spraying anisaldehyde-H_2SO_4 reagent); EI-MS *m/z* 220 $[M]^+$ (1), 205 (3), 187 (5), 177 (7), 161 (10), 149 (12), 135 (15), 121 (28), 109 (43), 93 (73), 79 (96), 69 (55), 55 (45), 43 (88), 41 (100); calcd for $C_{15}H_{24}O$ 220 g; ν_{max} 3070, 2950, 1620, 1465, 870 cm^{-1}; ^{1}H NMR: δ 4.86, 4.98 (2 *s*, 2H; H-15_a and H-15_b), 2.87 (*dd, J* = 4.6 Hz, 1H; H-5), 2.61 (*q, J* = 9.3 Hz, 1H; H-9), 2.33 (*ddd, J* = 4.6, 7.7 and 12.4 Hz, 1H; H-7a), 2.24 (*ddt, J* = 4.6, 7.7 and 9.3 Hz, 1H; H-6_b), 2.11 (*m*, 2H; H-3_b and H-7_b), 1.76 (*t, J* = 9.3 Hz, 1H; H-1), 1.65 (*m,* 3H; H-2_b and H-10), 1.43 (*m,* 1H; H-2_a), 1.30 (*m,* 1H; H-6_a), 1.20 (*s*, 3H; H-14), 1.01 (*s*, 3H; H-12), 0.98 (*m*, 1H; H-3_a), 0.98 (*s*, 3H; H-13); ^{13}CNMR: δ 151.9 (C-8), 112.7 (C-15), 63.7 (C-5), 59.8 (C-4) 50.9 (C-1), 48.7 (C-9), 39.8 (C-10), 39.2 (C-3), 34.0 (C-11), 31.6 (C-6), 30.2 (C-7), 29.9 (C-13), 27.2 (C-2), 21.6 (C-12), 17.0 (C-14).

Germacrene D (**3**): colorless oil; R_f = 0.20 ($AgNO_3$-impregnated silica-gel F_{254}, *n*-hexane:ethyl ether (98:2), three developments, visualization by spraying anisaldehyde-H_2SO_4 reagent); EI-MS *m/z* 204 $[M]^+$ (17), 161 (100), 147 (7), 133 (25), 105 (77), 91 (73), 79 (41), 55 (24), 41 (62), calcd for $C_{15}H_{24}$ 204 g; ν_{max} 2930, 1450, 985, 880 cm^{-1}; ^{1}H NMR: δ 5.78 (*d, J* = 15.7 Hz, 1H; H-5), 5.25 (*dd, J* = 10 and 15.9 Hz, 1H; H-6), 5.13 (*dd, J* = 4.3 and 10.1 Hz, 1H; H-1), 4.81 (*d, J* = 2.4 Hz, 1H; H-15_a), 4.72 (*d, J* = 1.7 Hz, 1H; H-15_b), 2.40 (*m*, 1H; H-3_a), 2.29 (*m,* 1H; H-2_a), 2.22 (*m,* 2H; H-9), 2.05 (*m*, 1H; H-3_b), 2.01 (*m*, 1H; H-7), 1.98 (*m*, 1H; H-2_b), 1.59 (*s*, 3H; H-14), 1.48 (*m*, 2H; H-8), 1.43 (*m,* 1H; H-11), 0.86 (*d, J* = 6.6 Hz, 3H; H-12), 0.81 (*d, J* = 6.6 Hz, 3H; H-13).

Antimicrobial activity

The antimicrobial activity was evaluated as described by Deuschle et al., 2007. The microorganisms tested were *Saphylococcus aureus* ATCC 25923, *Saccharomyces cerevisiae* ATCC 2601, *Pseudomonas aeruginos*a ATCC 27853, *Candida albicans* ATCC 90028, *Acinetobacter baumannii* and *Prototheca zopfii* (clinical isolates). Additionally, compound **1** was tested against *Candida tropicalis* ATCC 750 and compound **2** was evaluated against *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 10031. For compound **1** the tested concentrations ranged from 0.025 and 5 mg/mL while for compound **2** they varied between 53.62 and 1716 µg/mL.

RESULTS AND DISCUSSION

The structure of **1** was established as β-caryophyllene, a natural product isolated from several other *Senecio* species (Bohlmann & Zdero, 1982a; Bohlmann et al., 1981, 1986; Jakupovic et al., 1991; Kite & Smith, 1997; Mericli et al., 1989; Urzúa & Andrade, 2001). This compound displays antiinflamatory (Tambe et al., 1996), anticarcinogenic (Zheng et al., 1992) and cytotoxic (Kubo et al., 1996) activities. Besides, β-caryophyllene can autoxidize when air exposed to produce β-caryophyllene oxide, a compound with moderate allergenic activity (Sköld et al., 2006).

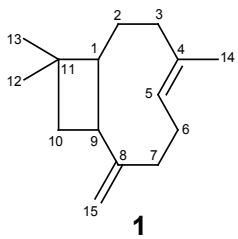
Compound **2** was proved to be β-caryophyllene oxide, which was also described for some *Senecio* species (Bohlmann et al., 1986; Jakupovic et al., 1991; Reina et al., 2002). This compound displays citotoxic (Kubo et al., 1996; Reina et al., 2002; Sibanda et al., 2004), antimalarial (Thebtaranonth et al., 1995), anticarcinogenic (Zheng et al., 1992), repellent against the mosquito *Anopheles gambiae* (Omolo et al., 2004), as well as antifungal properties (Yang et al., 2000). Although this substance is an artefact, it has been approved by the FDA as a food and cosmetic preservative and has been included by the European Council in the list of natural and synthetic flavouring substances (FDA, 1973). It appears to be tolerable, safe and toxic-free at the usual concentrations (Yang et al., 2000).

The structure of **3** was established as germacrene D, a hydrocarbon commonly found as plant constituent and considered to be a key intermediate in the biosynthesis of many sesquiterpenoids (Bülow & König, 2000; Prosser et al., 2004; Steliopoulos et al., 2002). This compound is present in several plant families, including Asteraceae (Mouzuraitis et al., 2002) and is frequently found in *Senecio* species (Bohlmann & Zdero, 1982b; Bohlmann et al., 1980, 1983, 1985; Deuschle, 2003; Deuschle et al., 2007; Francescato et al., 2003; Francescato, 2007; Murari 2007; Jakupovic et al., 1991). As biological activity is to emphasize it's ability to interact with insects and other organisms (Mozuraitis et al., 2002; Rostelien et al., 2000; Stranden et al., 2002). According to literature data, the content of germacrene D varies among plant species from pure (+)- or (-)-enantiomers to the racemate, controlled by separate synthases (Schmidt et al., 1998). Although this compound is present as its (-)-enantiomer in most higher plants, for several the occurrence of both enantiomers has been described (Bülow & König, 2000). Once the gas chromatography employed wasn't enantioselective and the optical rotation of compound 3 has not been measured, it remains unknown which enantiomer is present in *S. bonariensis*.

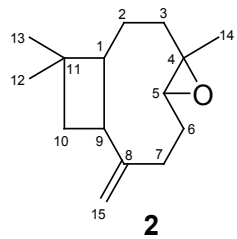
The three compounds have a relative close biosynthetic correlation as shown in Figure 1. From *trans,trans*-FPP, several enzymatic reactions occur, conducting to cyclizations, eliminations and subsequent rearrangements, originating several intermediates, as the germacrenyl- and humulyl-cations, which are responsible for the formation of different sesquiterpenoids, such as germacrene D and β-caryophyllene. The latter substance, through a process of autoxidation and degradation, following by cyclization and epoxidation, produces β-caryophyllene oxide.

The large genus *Senecio* is chemically and morphologically not very homogenous (Dupré et al., 1991), and there are still problems concerning the delimitation of species groups within the genus (Bohlmann et al., 1980). Thus, terpenoidic quinones and triterpenes are widespread in some species, while sesquiterpenes are very localized and poorly represented (Tundis et al., 2005). Otherwise, there are differences between succulent Senecio species and the other ones (Bohlmann et al., 1980, 1981). In our systematic studies with Brazilian indigenous *Senecio* species, we observed that sesquiterpenes are widespread among the analyzed plants. It seems that the most South-Brazilian *Senecio* species are rich in caryophyllane, germacrane-, eudesmane- and gauaiane-derivatives (Deuschle et al., 2007; Francescatto et al., 2003; Murari, 2007; Ghisalberti, 1994), which are formed form trans,trans-FPP (Bülow & König, 2000). However, the succulent species contain mainly bisabolane derivatives (Rücker et al., 1996, 1997), which are originated from *cis,trans*-FPP (Thol, 2006). These findings could be of chemotaxonomical significance and may be contribute to the botanical classification of these plants.

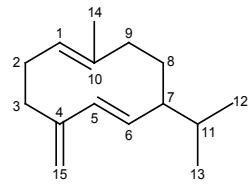
Compound **3** has its antimicrobial activity evaluated by an earlier work (Deuschle et al., 2007). Although the isolated sesquiterpernoids are often find in essential oils which presented antimicrobial activity, they were inactive at the tested concentrations.



1



2



3

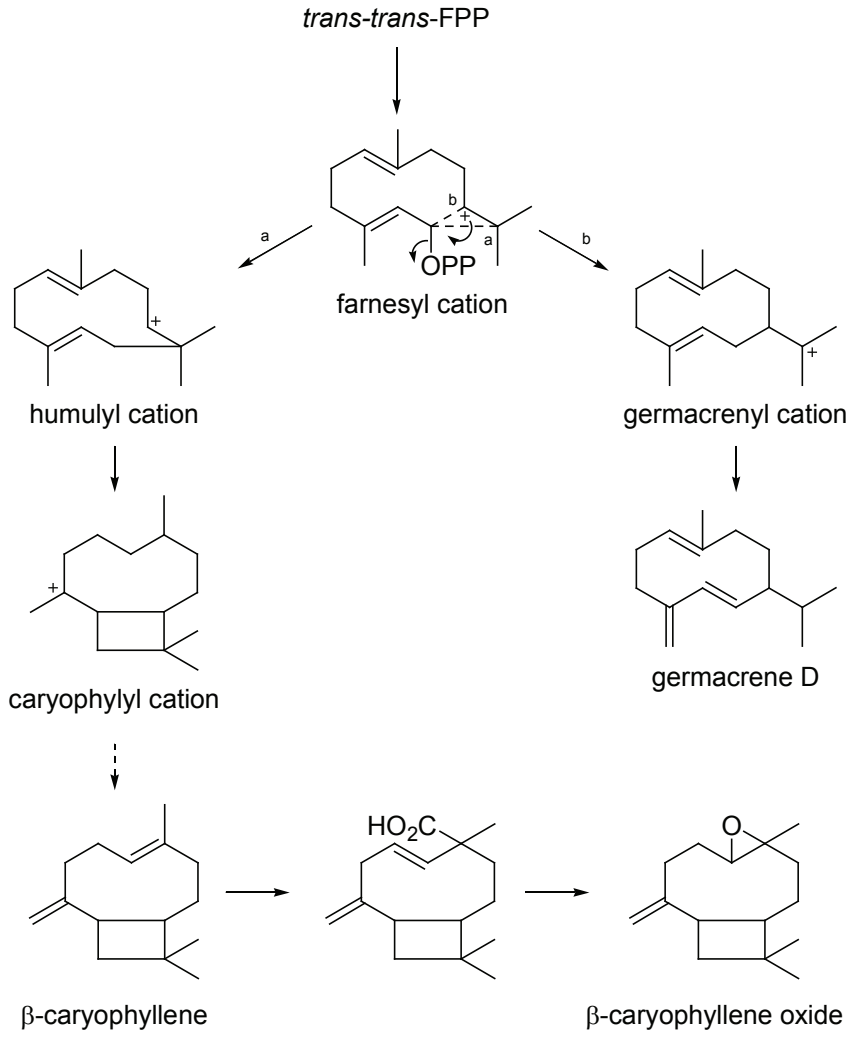


Figure 1. Biosynthesis of germacrene D and β-caryophyllene, as well as formation of caryophyllene oxide (adapted from Bülow & König, 2000; Fricke, 1999; Greenhagen, 2003; Prosser et al., 2002).

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