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Diterpene from *Baccharis trimera* with a relaxant effect on rat vascular smooth muscle

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Dedicated to Professor Dr. O.R. Gottlieb on the occasion of his 80th birthday

Abstract

A bioassay monitored fractionation of a chloroform extract from the aerial parts of *Baccharis trimera* yielded a mixture that blocked the Ca^{2+}-induced contractions of KCl- depolarized rat portal vein preparations. Pharmacological tests of two pure compounds isolated from the mixture revealed the dilactonic clerodane diterpene as the active compound.

Keywords: *Baccharis trimera*; Asteraceae; Portal vein; Dilactonic clerodane diterpene

1. Introduction

Baccharis trimera (Less.) DC. (Asteraceae) is a widespread South American plant known as "carqueja". Medicinal teas prepared from the flowering plant are used in folk medicine to treat gastrointestinal and liver diseases, angina, poor blood circulation, diabetes, and inflammatory processes (Corrêa, 1984; Sousa et al., 1991). Aqueous extracts prepared from the aerial parts of the plant were shown to decrease gastric acid secretion and gastric lesions induced by stress or by ethanol, in rats and mice (Gamberini et al., 1991; Lapa et al., 1992). The same extract also produced relaxation of the intestinal smooth musculature (Gamberini et al., 1991). Tests in rodents did not reveal toxic effects of the plant extracts (Gamberini et al, 1991; Lapa et al., 1992). Chemical studies revealed the presence of flavonoids and terpenes in *B. trimera* (Bohlmann and Zdero, 1969; Herz et al., 1977). The present study reports the relaxant effects produced by a dilactonic clerodane diterpene isolated from this species on the smooth musculature of rat portal vein preparations.

2. Results and discussion

The previously reported relaxant effect of the aqueous extract of *B. trimera* on the intestinal smooth musculature (Gamberini et al., 1991) was increased when the aerial parts of the plant were extracted with chloroform and then partitioned with water. LH-20 CC of the aqueous phase yielded 27 fractions that were monitored by TLC. The last two fractions were the most active. These observations suggested an intermediary polarity of the active compound.

A new chloroform extract was prepared from the aerial parts of the plant and fractionated on silica gel and Sephadex LH-20 yielding the water soluble, active fraction FA_1. Further purification of FA_1 on Sephadex LH-20 yielded the flavonoid eupatorin and a diterpene. The diterpene was identified by ^1H and ^{13}C NMR (DEPT, HMBC, HMQC, NOESY and GC–MS) as **1** (Herz et al., 1977, Kuroyanagi et al., 1985).

Both the diterpene **1** and eupatorin were tested on rat portal vein preparations depolarized with a 80 mM KCl Ca^{2+}-free physiological solution. Depolarization of the vascular smooth muscle cells with a high-KCl medium allows the influx of Ca^{+2} through L-type channels triggering a contractile response (Magnon et al., 1995).

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Cumulative addition of $CaCl_2$ (10 μM to 100 mM) to depolarized rat portal vein preparations caused concentration-related contractions of the smooth musculature with a mean effective concentration (EC_{50}) of 2.93 mM (geometric mean) (95% confidence limits: 2.52–3.63 mM). Incubation of FA_1 (300 μg/ml) shifted the concentration-response curves to $CaCl_2$ to the right 1.5-fold and decreased the maximum contraction by 32% (Fig. 1). At the same concentration, diterpene **1** (300 μg/ml) increased the EC_{50} of Ca^{2+} 4-fold and decreased the maximum contraction by 62%, while the isolated flavonoid was ineffective (Fig. 1).

Contraction of smooth muscles is proportional to both intracellular free calcium concentration released from the sarcoplasmic reticulum (Tribe et al., 1994), and to the influx of Ca^{+2} through calcium channels on the cell membrane (Hughes, 1995). Our results showed that diterpene **1** is the active principle of *B. trimera* blocking the vascular smooth muscle contractions induced by extracellular Ca^{2+} in KCl-depolarized preparations. This effect might correlate to the vasodilation and improvement of blood circulation referred in folk medicine. The mechanisms underlying the vascular effects of diterpene **1** are currently being examined.

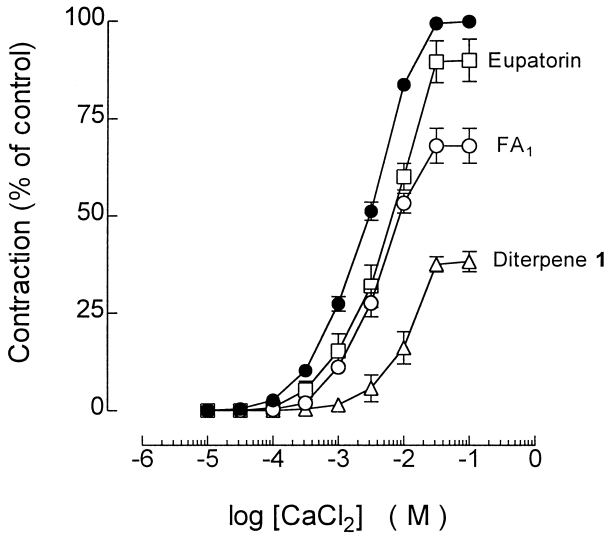


Fig. 1. Effects of fraction FA_1 (○, 300 μg/ml), diterpene **1** (Δ, 300 μg/ml) and the flavonoid eupatorin (□, 300 μg/ml), isolated from the chloroform extract of *Baccharis trimera,* on the calcium-induced isometric contraction of rat portal vein segments depolarized in 80 mM KCl- calcium free Krebs solution. Diterpene **1** and eupatorin were dissolved in Tween 2%. Control concentration-response curves to calcium (●) obtained in the absence and presence of the vehicle (Tween 2%) were not significantly different. The symbols and vertical bars are means ± S.E.M. of four to 15 preparations.

3. Experimental

3.1. Plant material

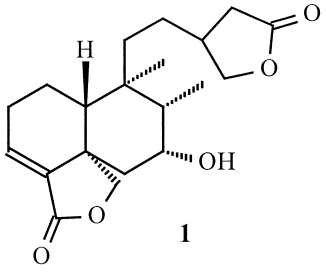
Baccharis trimera was cultivated from germplasm conserved in the Centro Nacional de Recursos Genéticos e Biotecnologia (CENARGEN) / Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), in Brasília, DF, and identified by Dr. Ladislau A. Skorupa who kindly provided the plant. The aerial parts of the flowering plant were dried at room temperature and stored in plastic bags protected against light and humidity.

3.2. General experimental procedures

Mps were uncorrected. Optical rotation was measured in $CHCl_3$ in a digital polarimeter JASCO DIP-370 (Na filter, $\lambda = 588$ nm); IR spectra were obtained from KBr pellets in a Perkin-Elmer infrared spectrometer model 1750; Elemental analysis was performed in a Perkin-Elmer elemental analyser model 2400 CHN. ^1H and ^{13}C NMR spectra were recorded on a 500 MHz Bruker DRX-500 instrument with tetramethylsilane (TMS) as an internal standard in $CDCl_3$, δ values were expressed in ppm. GC–MS was measured with a INCOS 50 Finnigan-MAT-quadrupole instrument (70 eV). HPLC column Shim-Pack, ODS (2500×4.6 mm id), flow rate 1.0 ml/min 100% ACN SPDM-10AVP detector (UV) diode array (Shimadzu). GC (HP-5890), capillary column HP5 5% phenyl methyl silicone, 30 m×0.32 mm; 0.5 μm, split 1:30, flow rate 6 ml/min, carrier gas He, automatic injector HP 767. Column chromatography was carried out with Aldrich silica gel (200–400 mesh) and Sephadex LH-20 (Pharmacia Biotech.).

3.3. Extraction and isolation

The dried powdered aerial parts of *Baccharis trimera* (50 g) were extracted using a Soxhlet apparatus with $CHCl_3$ (1 l) for 24 h. The chloroform extract was evaporated to dryness under red. pres. yielding a crude residue (1.6 g). Its subsequent chromatography on silica gel (200–400 mesh, Aldrich) using a CH_2Cl_2–MeOH gradient gave 31 fractions that were monitored by TLC silica gel, eluted with MeOH–$CHCl_3$–H_2O; 95:5:0.5. The combined active fractions 13–15 (FA_1, 400 mg) were repeatedly chromatographed on Sephadex LH-20, eluted with MeOH to yield the flavonoid eupatorin and diterpene **1**.



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3.4. Pharmacological studies

Depolarized portal vein in 80 mM KCl and no calcium nutritive solution contracted to bath application of $CaCl_2$. The portal veins isolated from female Wistar rats (180–250 g) were placed in a Krebs solution of the following composition (mM): NaCl 119.0, KCl 4.6; $MgCl_2$ 1.2; $NaHCO_3$ 15.0; $CaCl_2$ 1.5; NaH_2PO_4 1.2 and glucose 11.0, at 37°C and gassed with 95% O_2 and 5% CO_2. After rapid dissection segments of the portal vein (15 mm length) were mounted vertically under a resting tension of 1 g in a 3 ml organ bath containing Krebs solution. Isometric contractions of the organ were recorded using a force displacement transducer (Grass FT03) on an ''Ugo Basile'' recorder. After 60 min, the normal Krebs solution was replaced by a Ca-depleted high KCl (80 mM) solution and cumulative concentration-response curves were constructed to $CaCl_2$ (10 μM to 100 mM) prior to and after 15 min exposure to either fraction FA_1, diterpene **1** or eupatorin.

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References

Bohlmann, F., Zdero, C., 1969. Über neue Terpenderivate aus *Baccharis trimera*. Tetrahedron Letters 28, 2419–2421.

Corrêa, M.P., 1984. Dicionário das plantas úteis do Brasil e das exóticas cultivadas, Vol. I. Imprensa Nacional, Instituto Brasileiro de Desenvolvimento Florestal, Rio de Janeiro, (p. 74).

Gamberini, M.T., Skorupa, L.A., Souccar, C., Lapa, A.J., 1991. Inhibition of gastic secretion by a water extract from *Baccharis triptera* Mart. Memórias do Instituto Oswaldo Cruz 86 (Suppl. II), 137–139.

Herz, W., Pilotti, A.M., Söderholm, A.C., Shuhama, I.K., Vichnewski, W., 1977. New ent-clerodane-type diterpenoids from *Baccharis trimera*. Journal of Organic Chemistry 42 (24), 3913–3917.

Hughes, A.D., 1995. Calcium channels in vascular smooth muscle cells. Journal of Vascular Research 32, 353–370.

Kuroyanagi, M., Fujita, K., Kazaoka, M., Matsunoto, S., Ueno, A., Fukushima, S. et al., 1985. Studies on the constituents of *Baccharis genistelloides*. Chemical and Pharmaceutical Bulletin 33 (11), 5075–5078.

Lapa, A.J., Fischman, L.A., Gamberini, M.T., 1992. Inhibitors of gastric secretion from Brazilian folk medicinal plants. In: Capasso, F., Mascolo, N. (Eds.), Natural Drugs and the Digestive Tract. EMSI, Roma, pp. 63–68.

Magnon, M., Gallix, P., Cavero, I., 1995. Intervessel (arteries and veins) and heart/vessel selectives of therapeutically used calcium entry blockers: variable, vessel dependent indexes. Journal of Pharmacology and Experimental Therapeutics 275 (3), 1157–1166.

Sousa, M.P., Matos, N.E.O., Matos, M.F.J.A., Machado, M.I.L., Craveiro, A.A., 1991. Constituintes Químicos Ativos de Plantas Medicinais Brasileiras, Fortaleza, Edições UFC.

Tribe, R.M., Borin, M.L., Blaustein, M.P., 1994. Functionally and spatially distinct Ca^{2+} stores are revealed in cultured vascular smooth muscle cells. Proceedings of the National Academy of Sciences of the United States of America 91, 5908–5912.