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SHORT COMMUNICATION

ent-Kaur-16-en-19-oic acid, a KB Cells Cytotoxic Diterpenoid from *Elaeoselinum foetidum*

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Toxic and cytotoxic activities of the toxic plant *Elaeoselinum foetidum* (Apiaceae) were evaluated using the brine shrimp toxicity (BST) and KB cell cytotoxicity assays. The active chloroform extract was subjected to a bioactivity-directed fractionation, monitored by the BST assay, that led to the isolation of the diterpenoid *ent*-kaur-16-en-19-oic acid. This compound was potent against the brine shrimp (LC_{50} = 4.8 μg/mL) and KB cells (IC_{50} = 1.6 μg/mL). Copyright © 2002 John Wiley & Sons, Ltd.

Keywords: elaeoselinum foetidum; ent-kaur-16-en-19-oic acid; brine shrimp test; KB cells; cytotoxicity.

INTRODUCTION

Many plants that are known for their toxicity possess useful cytotoxic compounds (Kingston, 1992). *Elaeoselinum foetidum* L. Boiss (syn. *Thapsia foetida* L.) belongs to the family *Apiaceae* and grows in the Southwest of the Iberian Peninsula and the Northwest of Africa. The inhabitants of the rural areas of Cádiz, Spain reported toxicity cattle of this plant. Bioactivity-guided fractionation of the active chloroform extract using the brine shrimp toxicity test (Solis *et al.*, 1993) as a monitor assay yielded the compound *ent*-kaur-l6-en-19-oic acid (*ent*-**KA**). Herein we present results on the fractionation and the evaluation of the activity of *ent*- **KA** in the BST, KB cell cytotoxicity (Likhitwitayawuid *et al.*, 1993) and DNA–methyl green bioassays (Burres *et al.*, 1992).

MATERIALS AND METHODS

Plant material. Aerial parts of *E. foetidum* were collected in the region of Cádiz, Spain, in June 1999. A voucher specimen (Bustamante 8) was deposited at the Herbarium of Facultad de Ciencias, Universidad de Cádiz, Spain.

Plant extract. A chloroform extract was prepared by extracting 50 g of dry powdered plant material for 24 h at room temperature with chloroform. The extract was filtered and concentrated under reduced pressure at 43 °C

Brine shrimp toxicity assay. The brine shrimp toxicity (BST) assay was performed according to standard protocols (Solis *et al.*, 1993). The LC_{50} values in μg/mL were determined for the methanol extract, partitioned fractions, the pooled chromatographic fractions, and the isolated compound using a Finney probit analysis computer program (McLaughlin, 1993).

KB cell cytotoxicity. KB cell cytotoxicity was determined as described in Likhitwitayawuid *et al.* (1993) with modifications, using human oral epidermoid carcinoma (KB) provided by the University of Chicago at Illinois. Cells were typically grown to 90% confluence. Aliquots of 190 μL containing 5×10^4 cells/mL were added to the 96-well plates. The plates were incubated for 24 h at 37 °C in a CO_2 incubator. After this, 10 μL of serial dilutions of the samples were added to the wells, the plates were incubated for 48 h (37 °C, CO_2 atmosphere) and the absorption determined at 495 nm using sulphorhodamine B. A day 0 control was performed and the averaged value obtained with the day 0 control was subtracted. These values were then expressed as a percentage, relative to the solvent-treated control incubations, and IC_{50} values were calculated from a semi-log plot of the drug concentration against the percent of viable cells. Colchicine (Sigma) was used as a positive control (IC_{50} = 0.02 μg/mL).

DNA–methyl green bioassay (DNA–MG). Interaction with DNA was determined using 96-well microplates (Sigma, USA) as described in Burres *et al.* (1992) using the DNA–methyl green reagent, The decrease in the initial absorbance of each sample was read at 655 nm using a Biorad Microplate Model 450 Reader (Biorad,

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USA). Doxorubicine hydrochloride was used as a positive control (IC_{50} = 30 μg/mL). The activity was considered significant when three independent assays showed a 20% or greater inhibition.

Extraction and isolation. The bioactive chloroform extract (12.2 g) was supported over silica gel and fractionated by flash chromatography eluted initially with *n*-hexane, followed by a gradient of EtOAc up to 100% and, finally, methanol to give seven fractions. The bioactive second fraction (2.37 g, LC_{50} = 15 μg/mL) was further separated on a silica gel column eluted by n-hexane, *n*-hexane–EtOAc mixtures, EtOAc and methanol. The second of five combined fractions (1.19 g, LC_{50} = 17.4 μg/mL) showed the major activity. Purification of the aliquots of this fraction by HPLC (Merck-Hitachi L-7100 HPLC System with refraction index monitor; column LiChrosorb Si-60, 7 μm particle size, 1 × 25 cm; eluent *n*-hexane–EtOAc (4:l); flow rate 3 mL/min) afforded 987.5 mg of *ent*-**KA**, colourless prisms, mP, 179°–180°C.

RESULTS AND DISCUSSION

The chloroform extract of *E. foetidum* was tested in the BST and KB cell cytotoxicity bioassays, showing an LC_{50} = 25 μg/mL and an IC_{50} = 17 μg/mL respectively. This active extract was further submitted to bioactivity-guided fractionation by flash chromatography using the BST as a monitor assay. The active sub-fractions were purified by HPLC (Materials and Methods), yielding the diterpenoid *ent*-kaur-16-en-19-oic acid (*ent*-**KA** Fig. 1). The chemical structure was confirmed by spectral analysis (IR ^{1}H-NMR, ^{13}C-NMR, COSY, DEPT, HETCOR and MS) and comparison with published data (Ohno *et al.*, 1979). This compound, previously isolated from *E. foetidum* (Pinar *et al.*, 1983), has shown a wide range of biological activities, including cytotoxicity against MCF-7 cells (Fatope *et al.*, 1996); as an antifeedant (Lajide *et al.*, 1995), and antimicrobial, anthelminthic, insecticidal, inhibition of mitotic division, sporicidal (Padmaja *et al.*, 1995) and antinociceptive (Block *et al.*, 1998) activities. No information about its activity in the BST and KB cell cytotoxicity assays was found in the literature. In this work, the diterpene *ent*- **KA** showed toxicity in the BST assay with an LC_{50} = 4.8 μg/mL and cytotoxicity to KB cells with an IC_{50} = 1.6 μg/mL. The interaction of *ent*-**KA** with DNA was further studied using the **DNA–MG** test (Burres *et al.*, 1992). This assay detects the occurrence of compounds that interact with the DNA, but is not adequate for substances that bind covalently to DNA (Bonjean *et al.*, 1996). No interaction of *ent*-**KA** with DNA was observed with this method at final concentration of a 100 μg/mL Further studies are needed to understand the mechanism by which *ent*-**KA** exerts cytotoxicity towards KB and MCF-7 cells.

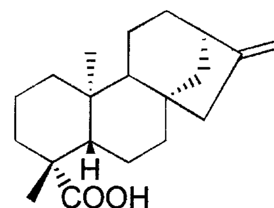

Figure 1. Chemical structure of *ent*-**KA**.

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