Semi-synthesis, structural elucidation and *in-vitro* anti-snake venom activity of irregular monoterpenes derivatives from *Baccharis trimera* (Asteraceae)

Manuel Minteguiaga[1,2,3]*, Ana María Torres[3], Gabriela Ricciardi[3], Eduardo Dellacassa[1], César A.N. Catalán[2]
****mminte@fq.edu.uy***

1. Laboratorio de Biotecnología de Aromas. Universidad de la República. Montevideo, Uruguay.
2. INQUINOA-CONICET. Universidad Nacional de Tucumán. S.M. Tucumán, Argentina.
3. Laboratorio de Productos Naturales. Universidad Nacional del Nordeste. Corrientes, Argentina.

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Introduction

Irregular monoterpenes are rare natural products found mainly in the Asteraceae family, whose skeleton does not follow the Ruzicka rule, which establishes a head-to-tail bond between the isoprene units [1]. Of the different types of terpenoids with reordered skeleton, those with an *o*-menthane skeleton are very rare, with the most relevant members being piquerols A and B from *Piqueria trinervia* Cav. and carquejol from *Baccharis trimera* (Less.) DC. (synonym: *B. genistelloides*) [2] (Figure 1) both plants belonging to the Asteraceae.

Irregular monoterpenes have received attention as research targets for bioactivities (e.g. insecticidal activity of pyrethrins) because of their ability to interact with biological systems [1,2].

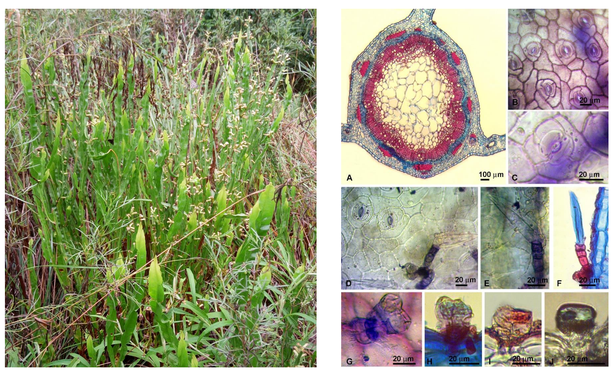

Figure 1: *B. trimera*: general appearance of the plant gowing wild in Uruguay, and anatomical features of the aerial parts: stomata, non-glandular and glandular trichomes (positive for essential oils).

Collaboration: Dra. María Inés Mercado and Dra. Graciela Ponessa (Fundación Miguel Lillo, S.M. Tucumán).

The interaction of natural or natural-derived products with proteins is extremely important for the inhibition of snake venom because certain compounds may act inhibiting enzymes such as phospholipases, proteases or coagulases neutralizing their effects *in vitro* and *in vivo* [3,4]. Although a *neo*-clerodane irregular diterpene isolated from *B. trimera* showed promising antiproteolytic and antihaemorrhagic properties [4], no reports on the activity of irregular monoterpenes against snake venoms were found.

Materials and Methods

Aerial parts of *B. trimera* at full flowering stage were collected at Paysandu (Uruguay) and extracted for it essential oil by steam distillation. The analysis of the oil composition was performed by GC-MS [5]. The main compound, carquejyl acetate (**1**) was isolated by column chromatography (CC) and several natural or semi-synthetic derivatives were obtained i.e., carquejol (**2**), carquejone (**3**), 2-isopropenyl-3-methylphenol ('carquejyl phenol', **4**) and 7,8-epoxy-carquejol (**5**) as shown in Scheme I. All products were purified by CC and their structures established by 1D and 2D ^{1}H-NMR, ^{13}C-NMR and MS. The rearrangement of carquejone (**3**) to isocarquejone (**6**) was evidenced in the silica gel stationary phase during CC. Products 2-4 and pure *B. trimera* essential oil, were tested by their *in-vitro* activity against the venoms of *Bothrops diporus* Cope and *Bothrops alternatus* (Duméril) Bibron & Dúmeril (Viperidae) through SDS-PAGE, hemolytic, proteolytic and coagulant inhibition assays as previously reported [3,4].

References

[1]. Ruzicka, L. **1953**. *Experientia.* 9(10), 357-396.
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Results

Essential Oil Composition

150 compounds were identified in the essential oil of *B. trimera.* The main of them were carquejyl acetate (**1**, 71.4%, **a**), palustrol (4.4%, **b**), β-pinene (2.9%, **c**), limonene (2.5%, **d**), *(E)*-β-ocimene (2.2%, **e**), germacrene D (1.8%, **f**), bicylogermacrene (1.2%, **g**) and carquejol (**2**., 0.5%, **h**). The GC-MS chromatogram is displayed in Figure 2.

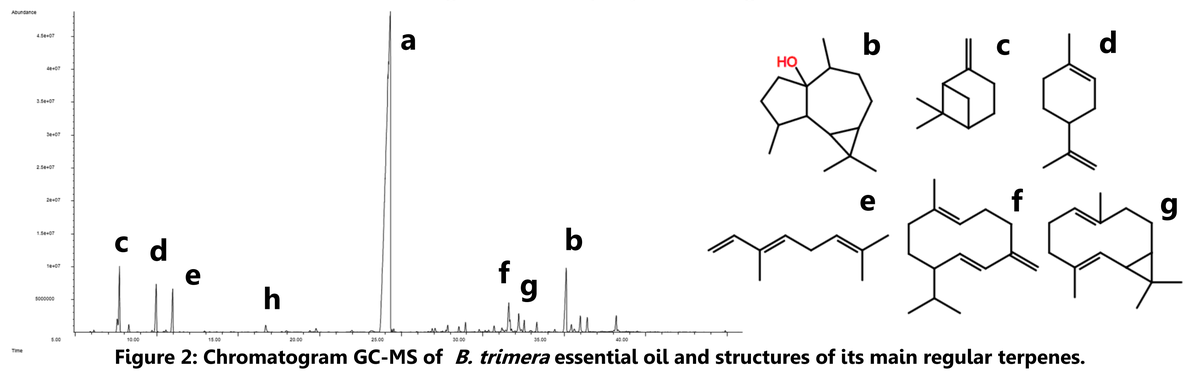
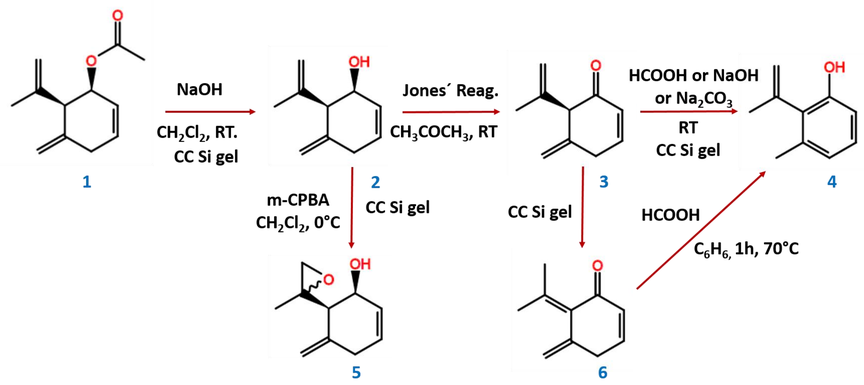


Figure 2: Chromatogram GC-MS of *B. trimera* essential oil and structures of its main regular terpenes.

Semi-synthesis

Products **1-6** were obtained and characterized spectroscopically.



Scheme 1: Semi-synthetic irregular monoterpenes derivatives obtained starting from *B. trimera* precursors.

Anti-snake venom activity

All products testes showed some degree of activity as it shown in Table 1. Carquejone (**3**) was the most active one against *B. diporus* and *B. alternatus* snake venoms (Table 1 and Figure 3).

Product	SDS-PAGE	PAI	HAI	CAI
EO	NA	Yes (+++)	NA	NA
2	NA	Yes (+++)	Yes (10.5%)	NA
3	Yes	Yes (+++)	Yes (50.0%)	Yes (20.4%)
4	Yes	Yes (+++)	NA	NA

Table 1: Anti-snake venom activity of *B. trimera* essential oil (EO) and natural and semi-synthetic derivatives products (according to Scheme 1) against *B. diporus* venom. References: SDS-PAGE: sodium dodecyl sulfate polyacrilamide gel electrophoresis; **PAI:** proteolytic activity inhibition; **HAI:** hemolytic activity inhibition; **CAI:** coagulant activity inhibition; **NA:** no activity.

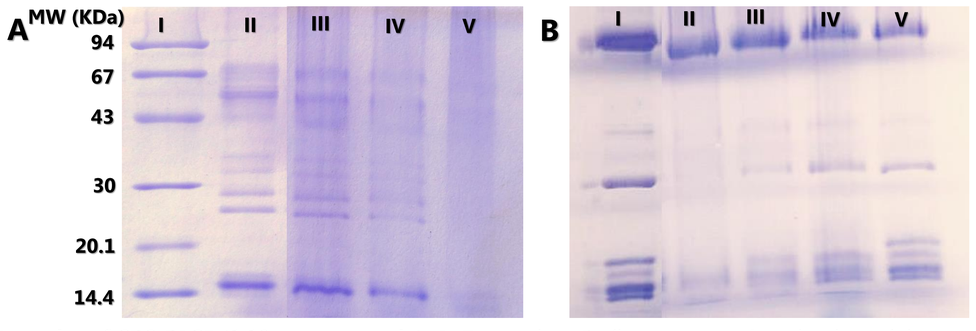


Figure 2: Examples of SDS-PAGE (1:10 venom:sample): **A:** Test againts *B. diporus* venom: **I:** molecular weight standard (BioRad); **II.** pure venom (V); **III. and IV.** essential oils of *B. punctulata* and *B. palustris* (control) + V; **V.** Carquejone (**3**) + V. **B:** Test against *B. alternatus* venom: **I.** pure venom (V); **II.** Carquejone (**3**)+ V; **III.** (*Z*)-lachnophyllum ester (control) + V; **IV. and V.** extracts in AcOEt of *B. articulata* and *B. trimera* (control) + V.

Conclusions

These results demonstrating that semi-syntethic irregular monoterpene carquejone is more active against bothropic venom than the natural products from *B. trimera*, highligthing the relevance of employing semi-synthetic approaches in the search of bioactive components.