Anti-Inflammatory Sesquiterpene Lactones from Genepy (*Artemisia umbelliformis* Lam.)

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Artemisia umbelliformis Lam. is an Alpine plant used to prepare the bitter liqueur 'genepy', granted a Geographical Indication status by EC. The wild chemotype of *A.umbelliformis* from Western Alps is characterized by the presence of the flavonoid eupatilin, of bitter sesquiterpene γ-lactones of the *cis* 8-olide type and by a high content of α- and ß-thujones. As the EU legislation set limits for thujones content in alcoholic beverages, *A.umbelliformis* chemotypes free of thujones were breeded. Phytochemical studies on these plants showed a replacement of C-8 *cis* lactones typical of the Western Alps plants by C-6 *trans*-lactones, and the presence of the sesterpene lactone genepolide (Appendino et al., 2009).

Some sesquiterpene lactones from a thujone-free Swiss chemotype were previously shown to exert *in vitro* effects suggestive of *in vivo* anti-inflammatory action. Thus, the topical antiphlogistic activity of a series of C-6 *trans* lactones from this chemotype (artemorine, costunolide, reynosine, santamarine, verlotorin, anhydroverlotorin, genepolide) was compared to that of C-8 *cis* lactones from the wild chemotype (umbellifolide, 5-deoxy-5-hydroperoxytelekin and 5-deoxy-5-hydroperoxyepitelekin) in the Croton oil-induced mouse ear dermatitis model (Tubaro et al., 1985), and compared to that of the steroidal and non steroidal reference drugs hydrocortisone and indomethacin. Six hours after dermatitis induction, all compounds exerted a dose-dependent anti-oedema effect, the most active being anhydroverlotorin (ID_{50}=0.35 μmol/cm^2) and genepolide (ID_{50}=0.40 μmol/cm^2), followed by 5-deoxy-5-hydroperoxyepitelekin, santamarine, 5-deoxy-5-hydroperoxytelekin and costunolide (ID_{50}=0.54-0.73 μmol/cm^2). The effect of anhydroverlotorin and genepolide was comparable to that of indomethacin (ID_{50}=0.26 μmol/cm^2) and only one order of magnitude lower than that of hydrocortisone (ID_{50}=0.03 μmol/cm^2). In addition, their overall anti-oedema effect (0.4 μmol/cm^2) up to 48h was intermediate between that of an equimolar dose of indomethacin or hydrocortisone (0.04 μmol/cm^2), with a profile similar to that of the steroid. The two compounds reduced also the leukocytes infiltrate, similarly to the reference drugs.

To identify the mechanisms of action, all compounds were evaluated for their effect on the transcription factor NF-κB in Jurkat T cells stimulated with tumor necrosis factor-α(TNF-α) or phorbol myristate acetate (PMA). Up to 100 μM, anhydroverlotorin, costunolide, 5-deoxy-5-hydroperoxyepitelekin, 5-deoxy-5-hydroperoxytelekin and umbellifolide inhibited both TNF-α and PMA-induced NF-κB with an IC_{50}≤20 μM. Santamarine, reynosine and verlotorine were less active, while genepolide was inactive. Thus, the potency as NF-κB inhibitors does not parallel the *in vivo* antiphlogistic activity. Anhydroverlotorin and genepolide were also evaluated for their antioxidant effect in skin HaCaT keratinocytes. However, no protective effect on reactive oxygen species (ROS) production and cytotoxicity induced by 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) or hydrogen peroxide were observed, except a moderate but significant reduction of AAPH-induced ROS production at 1 μM. These results on anhydroverlotorin, a twofold Michael acceptor, are surprising as, due to their electrophilicity, exomethylene lactones are known activators of the Nrf2 transcription factor-mediated antioxidant responses (Fischedick et al., 2012).

Taken together, our data demonstrate the *in vivo* anti-inflammatory activity of sesquiterpene lactones from *A.umbelliformis*, with the most active compounds being anhydroverlotorin and genepolide. While anhydroverlotorin is a Michael acceptor and an inhibitor of NF-κB, the activity of genepolide, a non electrophilic compound, is clearly related to a different mechanism.

Appendino et al. (2009). *J. Nat. Prod.* 72, 340-4.
Fischedick et al. (2012) *Planta Med.* 78, 1725-30
Tubaro et al. (1985). *Agents Actions* 17, 347-9.