A023 *In vivo* anti-inflammatory activity of *Isatis tinctoria* extracts and tryptanthrin

M.C. Recio [a], M. Hamburger [b] and J.L. Ríos [a]
[a] Department of Pharmacology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, E-46100 Burjassot, Spain. [b] Institute of Pharmacy, University of Jena, Semmelweisstrasse 10, D-07743 Jena, Germany.

Isatis tinctoria L. (Brassicaceae) is an old European and Chinese dye plant and medicinal herb which has been used for centuries in various inflammatory ailments. A broad *in vitro* pharmacological screening against 20 clinically relevant targets confirmed a promising anti-inflammatory profile of a dichloromethane (DCM) leaf extract. Tryptanthrin was subsequently identified as the extract's cyclooxygenase-2 inhibitory principle (1). The compound strongly inhibited COX-2 and 5-LOX catalyzed eicosanoid synthesis in various cellular models and in isolated enzymes (2).

In vivo activities of a DCM extract, a supercritical CO_2 (SFE) extract and tryptanthrin were assessed in the TPA-induced mouse ear oedema and in carragenan-induced mouse paw oedema as models for acute inflammation. Statistical signification was analyzed by Dunnett's *t*-test. Topical administration of extracts (0.5 mg/ear) inhibited significantly the ear oedema (32 % for SFE extract, 62 % for DCM extract). Oral administration (100 mg/kg for SFE extract, 125 mg/kg for DCM extract) inhibited the ear oedema by 37 % and 33 %, respectively, whereas effects of indomethacin (10 mg/kg) and tryptanthrin (70 mg/kg) were not significant in this application route.

In the mouse paw oedema, the extracts showed dose dependent inhibition which was strongest between 1 and 3 h after administration. The ED_{50}'s were 78 mg/kg (SFE extract) and 165 mg/kg (DCM extract). Indomethacin (10 mg/kg) showed maximal inhibition (65 %) after 3 h. No clear dose-effect relationship was found for tryptanthrin at doses up to 70 mg/kg. The weak activity of the purified compound may be due to its poor solubility, resulting in low cutaneous penetration and oral bioavailability. Additionally, the extracts appear to contain additional active principles. Their anti-inflammatory properties may hence be the sum of the multiple pharmacological activities discovered in the initial pharmacological profiling.

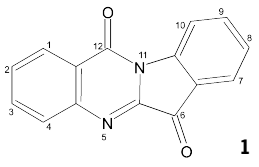
References: 1. Danz, H., et al. (2001), Planta Med. 67: 411-416. **2.** Danz, H., et al. (2002), submitted.

A024 Synthesis of d-tryptanthrin as an internal standard for quantitative LC – MS analysis

C. Oberthür , B. Hoffmann and M. Hamburger
Institute of Pharmacy, University of Jena, Semmelweisstraße 10, D-07743 Jena, Germany.

The indolo[2,1-*b*]-quinazoline alkaloid tryptanthrin (**1**) was recently identified as the COX-2 inhibitory principle in the ancient dye and medicinal plant *Isatis tinctoria* L. (Brassicaceae) (1). In cell based assays as well as with the isolated cyclooxygenases-1 and -2, **1** showed potent and highly selective inhibition of the COX-2 isoenzyme and a strong inhibition of LTB_4 release from human granulocytes. A quantitative ESI LC-MS assay for tryptanthrin in plant material and extracts hase been recently published (2). The use of an external calibration, however, requires frequent calibration of the MS detector. Anticipating future needs for tryptanthrin analysis in biological samples, the use of an internal standard was required.

In MS, isotope-labelled standards, i.e. *d*-tryptanthrin, are the preferred choice. Initial attempts to prepare *d*-tryptanthrin by deuterium exchange in **1** (*d*-TFA or D_3PO_4 x BF_3, 6d, 80°C) were not successful. The compound was finally prepared in three steps starting from d_5–aniline via *d*–isonitrosoacetanilide and *d*-isatin. The overall yield was 8,7%. The reaction of d_4–isonitrosoacetanilide to d_4–isatin proved to be critical with respect to hydrogen exchange, but its extent was minimized by the use of deuterated solvents. The isotope purity of the final product was determined by ESIMS (d_8–tryptanthrin 77,5%; d_7–tryptanthrin 20,7%, d_6-tryptanthrin 1,8%, $[M+H]^+$ ions). Possible positions for back exchange in d-tryptanthrin are at C-2, C-4, C-8 and C-10, as determined by residual signals in the ^1H – NMR spectra (3).



1

References: 1. Danz, H. et al. (2001) Plant. Med. 67: 411-416. **2.** Danz, H., et al. (2002) Plant Med. 68: 152-157. **3.** Oberthür, C., et al. (2002) Pharmazie, in press.