Synthesis of derivates of chamazulene carboxylic acid as potential inhibitors of inflammation via interaction with CB receptors

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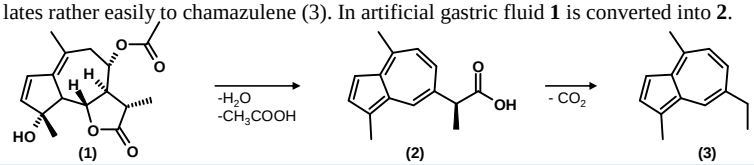
1. Introduction

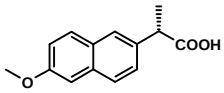
Both Matricaria recutita and Achillea millefolium contain proazulenic sesquiterpene lactones. One of them is matricin (1). The intensive dark blue azulene chamazulene carboxylic acid (CCA) (2) is a degradation product of **1**. **2** is unstable and decarboxylates rather easily to chamazulene (3). In artificial gastric fluid **1** is converted into **2**.




Matricaria recutita

2 is a natural profen. Its constitution is similar to other profens like naproxen and ibuprofen, viz. a propionic acid moiety linked to an aromatic system. Just like eutomers of synthetic profens, **2** is S-configurated. **2** was shown to inhibit COX-2 but not COX-1 and to have anti-inflammatory effects in animal models when both locally and topically applied.

After oral administration of **1** in human volunteers plasma levels of **2** in the micromolar range were found.[1]


(S)-Naproxen

2. Extraction of CCA

We use Achillea millefolium rather than Matricaria flowers for the extraction of **2** because its flowers are not covered by a cuticular wax, yielding fatty acids that are difficult to separate from **2**. After $CHCl_3$ extraction, **2** is generated through alkaline hydrolysis and further purified by liquid-liquid extraction. At first **2** remains as an anion in the aqueous phase. After acidification it is extracted into the organic phase and purified by MPLC on normal-phase silica gel (eluent: C_6H_{14}: $(H_3C)_3C\text{-}O\text{-}CH_3$: CH_3OH 65:30:5).[2]

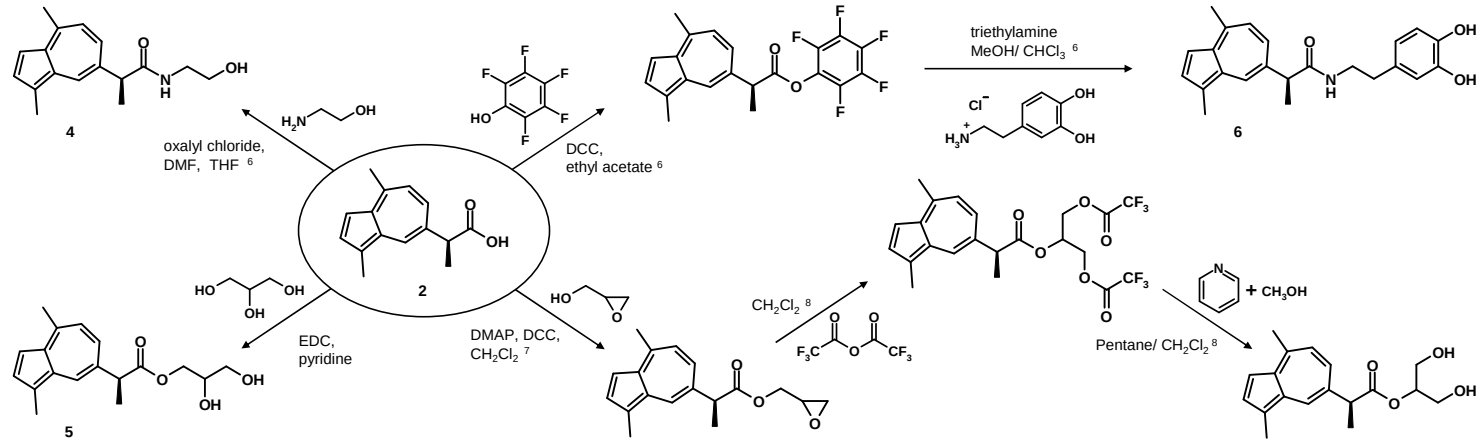

Chamazulene carboxylic acid

3. Endocannabinoids

Not only the cyclooxygenases COX-1 and COX-2 are involved in the process of inflammation, but also other players such as the endocannabinoid system. The latter consists of two G-protein-coupled receptors with – so far - two subtypes (CB1, CB2), fatty acid amide hydrolase and the anandamide transporter. In the last years, it was demonstrated that endogeneous derivatives of arachidonic acid – both esters and amides – were ligands at the CB receptors.[3] Arachidonic acid is a ligand of both COX enzymes. Thus there is a striking connection between both systems.

The CB2 receptor is mainly expressed in cells which are involved in the process of inflammation whereas the CB1 receptor is expressed in the brain. It was shown that SR144528, a CB2 receptor *antagonist*, inhibited the TPA-induced mouse ear oedema. WIN55212-2, an unspecific CB receptor *agonist*, also prevented the swelling provoked by TPA.[4,5] In order to elucidate the possible involvement of the anti-inflammatory CCA in the endocannabinoid system, we prepared possible metabolites of **2**, viz. **4**, **5**, **6**, and **7**. Compounds **4** and **7** might exert an effect through binding at CB receptors similar to anandamide (N-arachidonoylethanolamine) and 2-arachidonoylglycerol.

4. Syntheses



DMF dimethylformamide, THF tetrahydrofuran, DCC dicyclohexycarbodiimide, DMAP 4-(dimethylamino)pyridine, EDC 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

5. TPA-induced mouse ear oedema

12-O-Tetradecanoylphorbol-13-acetat, TPA, is an irritant and cocarcinogen naturally occurring in Croton tiglium L. (Euphorbiaceae). TPA causes an acute inflammation when it is topically applied. Six hours after the application the maximum is reached. 48 hours later the pinna thickness has reached almost its initial value and the inflammation has abated.

It was stated that tumour promoting substances provoke an ear oedema in mice. Furthermore it was discovered that tumour inhibiting substances reduce the development of the TPA-induced mouse ear oedema. By means of this test a substance can be tested for anti-inflammatory and potential tumour inhibiting effects at the same time.[9]

The compounds are tested topically. Using a microlitre pipette the solution is applied on the inner surface of the pinna 30 minutes prior to the TPA-application. On each day there is one control group treated with the mere solvent. We measure the increase and decrease of the thickness of the ear pinna with a calliper 3, 6, 24 and 48 hours after TPA-application starting with a gauging before any application. With this method we can monitor the development of the oedema in each animal.

6. Outlook

Compounds **4** to **7** will be tested in the TPA-induced mouse ear oedema. If they inhibit the oedema, of course this does not proof that these substances indeed interact with one of the CB receptors. For this, the active compounds have to be tested in an in vitro assay of the two receptors.

Since **2** is rather unstable, it would be expedient to test more stable derivatives of **2** if they have a similar effect.

2 is a natural product. We evaluate possible synthetic routes to **2** and related azulenes. The latter will also be tested topically in the TPA-induced mouse ear oedema.

7. Literature

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