Pharmacological activities and phytochemistry of various plant containing coumarin derivatives

Mohammad Asif
Department of Pharmacy, GRD(PG) Institute of Management & Technology, 248009, Dehradun, (Uttarakhand), India
*Corresponding author’s E-mail: asif321@gmail.com

Article type: Review article
Article history: Accepted January 2015
August 2015 Issue
Keywords: Pharmacological activities, Phytochemistry, Anti-inflammatory, Antimicrobial, Anti-cough, Anti-arthritic, Ethnopharmacological, Coumarins, Analgesia properties, Anti-inflammatory, Anti-microbial activities

Abstract
The physiological effects of natural coumarins are extensive. Humans are exposed to them in everyday life. The ethnopharmacological and biological properties of some natural coumarins have been investigated. These coumarins and furanocoumarins showed anti-inflammatory, antimicrobial, anti-cough and colds and anti-arthritis by the presence of furanocoumarins (psoralen) and xanthotoxin as defensive compounds. Coumarins are considered as phytoalexins as defence substances. The phototoxicity of linear furanocoumarins (psoralens) has been useful property, this psoralen and long wave ultraviolet treatment has been used for psoriasis. The osthol, a simple coumarin from Angelica archangelica, is useful compound for investigations on ligand-receptor interactions and for receptor-mediated regulations of intracellular free calcium concentrations. Coumarins can be beneficial for the plants themselves as natural biocontrolling antipathogenic compounds and for humans as remedy for hyperproliferative skin diseases. The coumarin-containing plants are valuable as dietary supplements on the basis of their mild antimicrobial and anti-inflammatory effects. The ability of dicoumarol to inhibit blood clotting and warfarin is used as an anticoagulant. Several biological activities have been reported in natural-occurring coumarins, from photo sensitizers to vasodilation. Recently, the interest has been given to synthetic coumarin derivatives, such as fluorinated and 1-azo coumarins, which displayed moderate analgesia properties, anti-inflammatory and anti-microbial activities.

Capsule Summary: The ethnopharmacological and biological properties of some natural coumarins derivatives were reviewed and coumarins and furanocoumarins have considerable anti-inflammatory, antimicrobial, anti-cough and anti-arthritis and might be potential candidates for pharmacological applications.

INTRODUCTION

Natural products are mainly secondary metabolites, produced by organisms in response to external stimuli such as nutritional changes, infection and competition (Cotton, 1996; Strohl, 2000). Natural products produced by plants, fungi, bacteria, insects and animals have been isolated as biologically active pharmacophores. Approximately one-third of the drugs in the world is natural products or their derivatives. Moreover, natural products are widely used in the pharmaceutical industry for their broad structural diversity and wide range of biological activities (Asif, 2015a-f; Ashraf et al., 2015; Harvey, 1999; Verpoorte 1989; Verpoorte, 2000). The major advantage of natural products for random screening is the structural diversity provided by natural products, which is greater than provided by most available combinatorial approaches based on heterocyclic compounds (Claeson and Bohlin, 1997; Harvey, 1999). The natural products will continue to offer novel leads for novel therapeutic agents, if the natural products are available for screening (Cotton, 1996). The ethnobotanical approach is based
on the traditional knowledge of medicinal plant use (Cox 1994). These compounds have made many plants useful also for humans for instance as spices, medicines etc. Natural coumarins, like other unsaturated lactones, may exert various effects on living organisms, both in plants and in animals. In view of their established low toxicity, relative cheapness, presence in the diet and occurrence in various herbal remedies, it appears important to evaluate the properties and applications of coumarins further utilising an ethnobotanical approach.

Coumarins: Coumarins are derivatives of 5,6-benzo-2-pirone (α-chromone) (with OH, OCH₃ or CH₃ substituents on the benzoic ring). The simple coumarins, C-prenylated and O-prenylated forms exist. As derivatives of simple coumarins, other compounds are known, such as furanocoumarins, which include a furanic ring, linear pyranocoumarins, angular pyranocoumarins, dimeric coumarins, of which dicoumarol is an example and also furanochromones. Historically, the ability of dicoumarol to inhibit blood clotting, that later led to the development of the anticoagulant drug warfarin, was the first call to this class of compounds’ biological properties. Several biological activities have been reported in natural-occurring coumarins, from photo sensitizers to vasodilatation. Recently, the interest has been given to synthetic derivatives of coumarins, such as fluorinated and 1-azo coumarins, which displayed moderate analgesia properties, and excellent anti-inflammatory and anti-microbial activities (Kalkhambkar et al. 2008). (Pereira et al. 2009).

Botanical aspects

Coumarins are found free or as heterosides in many dicotyledonous families, including the Apiaceae, Asteraceae, Fabiaceae, Moraceae, Rosaceae, Rubiaceae, Rutaceae and Solanaceae (Weinmann, 1997; Matern et al, 1999). Many monocotyledonous plants, especially the Gramineae and orchids, also contain large amounts of coumarins. Although mainly synthesised in the leaves, coumarins occur at the highest levels in the fruits, followed by the roots and stems. In addition, seasonal changes and environmental conditions may affect the occurrence in various parts of the plant. The distribution of biologically active coumarins in a wide range of plants seems to correlate with their ability to act as phytoalexins, i.e. they are formed as a response to traumatic injury, during the wilting process, by plant diseases or through drying, they accumulate on the surface of the leaves, fruits and seeds, and they inhibit the growth and sporulation of fungal plant pathogens and act as repellents against beetles and other terrestrial invertebrates (Weinmann, 1997; Matern et al, 1999). Coumarins are leached from the roots of some plants, such as wild Avena, into the soil, where they provide a defence tool against hostile micro-organisms. Coumarins are also active in plant metabolism, taking part in growth regulation (Weinmann, 1997; Matern et al, 1999). In particular furanocoumarins, are known to inhibit root tip growth and seem to induce membrane disturbances, and their excretion on seed surfaces might be a means to delay germination (Hålvi, 1988).

Ethnobotany/Ethnopharmacology

One part of ethnobotany, ethnopharmacology is considered as the scientific evaluation of traditional medicinal plants. (Cotton, 1996). Several members of the plant families Apiaceae and Rutaceae are used as spices and vegetables in human nutrition or for medicinal purposes. A. podagraria, A. graveolens, A. archangelica, L. officinale, P. crispum and R. graveolens are known as spices and vegetables, their medicinal uses as well as those of P. palustris’. The most common use of coumarin containing species seems to be different kinds of gastric disorders (Ziyyat et al, 1997; Tunali et al, 1999; Hsiao et al, 1998; Hänsel et al, 1994).

TRADITIONAL USE OF COUMARIN CONTAINING PLANTS AS DRUGS

Coumarin containing plants were tested for their antimicrobial and phototoxic activities. As the traditional uses presented and does not correlate directly to these activities, results of the coumarins found in these plants with respect to their relevance in the traditional use of these plants are discussed. Aegopodium podagraria showed only modest activity. Pure coumarins found in it, angelicin and aperin, failed to show any anti-inflammatory activity, and the total coumarin content was low, therefore the traditional use described in the literature as a remedy for rheumatism is not likely to be explained with the presence of coumarins in this plant. Anethum graveolens contains, among other compounds, xanthotoxin, scopoletin, umbelliferone and bergapten. Xanthotoxin was active in the anti-inflammatory tests. Xanthotoxin, scopoletin and umbelliferone exhibited strong inhibition against the plant pathogen Fusarium culmorum. Phototoxicity of the linear furanocoumarins bergapten and xanthotoxin was proven with the new Artemia salina method. However, extracts of A. graveolens had only a modest activity in the antimicrobial tests and were inactive in the phototoxicity which might be attributed to the low coumarin content. Angelica archangelica was the second richest in total coumarin content, it contains for instance psoralen and xanthotoxin, active in our anti-inflammatory tests, which might, at least in part, explain its ethnobotanical use as a remedy for cough, colds and rheumatism. The strong phototoxic effect seen with A. archangelica can be ascribed to the presence of psoralen (bergapten, psoralen, xanthotoxin). The antimicrobial effect of A. archangelica was more pronounced against the plant pathogens. Levisticum officinale contains coumarins, aperin, bergapten, coumarin and umbelliferone, were not active in the anti-inflammatory test, and the amount of coumarins was low, the antitussive and antiedematous properties utilised in the folk medicine are likely to be due to compounds of other type. However, a clear dependence of the phototoxicity on the coumarin content was proven: the phototoxicity of L. officinale observed before the flowering period was abolished as the coumarin content in the leaves decreased after flowering. Methanol extract of Petroselinum crispum possessed inhibitory activity against the widest spectrum of microbes, and furthermore, it contains xanthotoxin, active in our anti-inflammatory tests, which might
partly explain its ethnobotanical use as a remedy for cough, colds and rheumatism. Xanthotoxin was not active in calcium flux tests suggesting that other mechanisms may explain its use as a remedy for hypertension. Although the total amount of coumarins in *P. crispm* was not high, the compounds present (bergapten and xanthotoxin) were highly phototoxic ones. *Peucedanum palustre* was the richest in total coumarin content, the coumarins e.g. bergapten and peucedanin, were not active in the anti-inflammatory test, the folk medicinal use as a treatment for cough, rheumatism, fever and colds is therefore likely due to other substances and/or mechanisms. Additionally, the antimicrobial effects of the leaf extract were more specifically against plant pathogenic fungi. Strongly phototoxic coumarins seem to appear on the later stages of plant development since the phototoxic effect strengthen although the amount of coumarins in leaves decreases during the vegetative period. Xanthotoxin and psoralen found in *Ruta graveolens* might, at least to some extent, explain its ethnobotanical use as a remedy for cough, colds and rheumatism. This was further supported by the inhibitory activity of the leaf extract against the widest spectrum of microbes which might, at least partly, be addressed to xanthotoxin, umbelliferone, herniarin and scopoletin present in this plant, especially the latter two. Besides phototoxic, *R. graveolens* was also toxic to *A. salina* larvae.

**Phytochemistry of coumarins**

Coumarins owe their class name to ‘coumarou’, the vernacular name of the tonka bean (*Dipteryx odorata*, Fabaceae), from which coumarin itself was isolated (Bruneton, 1999). Coumarins belong to a group compounds known as the benzopyrones, all of which consist of a benzene ring joined to a pyrone. Coumarin and the other members of the coumarin family are benzo-a-pyrones, while the other main members of the benzopyrone group-the flavonoids-contain the γ-pyrene group (Keating and O‘Kennedy, 1997). Coumarins may also be found in nature in combination with sugars, as glycosides. The coumarins can be roughly categorised as follows (Murray et al., 1982). Simple-these are the hydroxylated, alkoxylated and alkylated derivatives of the parent compound, coumarin, along with their glycosides

**Furanocoumarins**

These compounds consist of a five-membered furan ring attached to the coumarin nucleus, divided to linear and angular types with substituents at one or both of the remaining benzenoid positions

**Pyranocoumarins**

members of this group are analogous to the furanocoumarins, but contain a six-membered ring coumarins substituted in the pyrone ring. Like other phenylpropanoids, coumarins arise from the metabolism of phenylalanine via a cinnamic acid, p-coumaric acid (Bruneton, 1999; Matern et al., 1999). The specificity of the process resides in the 2'-hydroxylation, next comes the photocatalysed isomerisation of the double bond followed by spontaneous lactonisation. In some rare cases, glucosylation of cinnamic acid occurs, precluding lactonisation. In such cases, coumarin only arises after tissue injury and enzymatic hydrolysis. The formation of di- and trihydroxycoumarins and of their ethers involves the hydroxylation of umbelliferone rather than the lactonisation of the corresponding cinnamic acids. Prenylation of the benzene ring by dimethylallyl pyrophosphate in the 6-position of a 7-hydroxycoumarin yields the so-called linear furano- and pyranocoumarins, in the 8-position it affords the angular homologues. The formation of furanocoumarins includes two successive steps: stereospecific oxidation in the 4'-position and elimination of the hydroxyisopropyl residue in the 5'-position by retroaldol condensation. Substitution in the 5- or 8-position or in both positions of furanocoumarins occurs later and is catalysed by oxidases and O-methyltransferases. The primary site of synthesis of coumarins is suggested to be the young, actively growing leaves, with stems and roots playing a comparatively minor role (Murray et al., 1982). However, one should not forget the possibility of species and compound variation, for example furanocoumarins in *Pastinaca sativa* are formed in the fruits where they also accumulate, and furanocoumarins in *Angelica archangelica* are formed in the leaves with the exception of ostenol, a simple coumarin, which is probably formed in the roots.

**BIOLOGICAL EFFECTS OF PLANT COUMARINS**

Coumarins have a variety of bioactivities including anticoagulant, estrogenic, dermal photosensitising, antimicrobial, vasodilator, molluscacidal, antihelminthic, sedative and hypnotic, analgesic and hypothermic activity (O‘kennedy and Thornes, 1997). Recent reports of the biological effects of coumarins (Chen et al., 1995; Okada et al., 1995; Lino et al., 1997; Hiermann and Schlantl, 1998; Hsiao et al., 1998; Garciaargaez et al., 2000; Sardari et al., 1999; Hoult et al., 1994; Liu et al., 1998; Resch et al., 1998).

**Anti-inflammatory activity**

Inflammation, starting from the description of the main four signs of inflammation, redness, swelling, heat, pain and loss of function, and ending to 'a multi-mediated phenomenon, of a pattern type in which all mediators would come and go at the appropriate moment to play their roles in increasing vascular permeability, attracting leukocytes, producing pain, local oedema and necrosis, in which the predominance of any one would be fortuitous or depending on its specific capabilities of\ producing symptoms, some directly, some indirectly, some by potentiating or by releasing other agents' (Rocha e Silva 1994). A controversy about the activity of the coumarins as anti-inflammatory agents exists, since some authors reported (Hoult and Payá, 1996) that coumarins do not exert potent activity in conventional short-term tests. Nevertheless, various coumarins have been reported to possess anti-inflammatory activity. The inflammation releases of several inflammatory mediators such as histamine, serotonin, bradykinin and prostaglandins (Lino et al., 1997). Non-steroidal anti-inflammatory drugs (NSAID) block the synthesis of prostaglandins by inhibiting cyclooxygenase (COX). The COX and 5-lipoxygenase (5-LO) catalyse peroxidation of arachidonic acid, and polyphenols like coumarins and flavonoids
might be expected to interfere with this process (Hoult et al., 1994b). Actually, fraxetin, esculetin, 4-methylesculetin, daphnetin and 4-methyl-daphnetin inhibited generation of leukotriene B4 (a 5-LO product) (Hoult et al., 1994a). Coumarin and umbelliferone were found to have a mechanism of action similar to NSAID in a carrageenan-induced inflammation, and the effect lasted for at least 3 h, which is the time for the maximum effect of carrageenan. Coumarin was also effective in the rat paw oedema induced by dextran. Osthol, isolated from Angelica archangelica, A. pubescens f. biserrata and Atractyloides lancea, turned out to be a selective inhibitor of 5-LO in vitro (Roos et al., 1997; Liu et al., 1998; Resch et al., 1998). Since 5-LO is activated by calcium influx, this effect was suggested to be due to its calcium antagonistic properties (Härmälä et al., 1992). Seselin from the aerial parts of Decatropis bicolor was active in the carrageenan-induced inflammation assay in rats. Carrageenan-induced rat paw oedema has been inhibited also by ethanol extract of the roots of Peucedanum ostruthium (Hiermann and Schlantl, 1998), 6-(3-carboxybut-2-enyl)-7-hydroxycoumarin being the most important anti-inflammatory compound in the plant. Carrageenan-induced inflammation was also suppressed by seselin isolated from Seseli indicum (Tandan et al., 1990) and by ethanol extract of the aerial parts of Ruta chalepensis (Al-said et al., 1990). Columbianadin, columbianetin acetate, bergapten and umbelliferone isolated from Angelica pubescens demonstrated both anti-inflammatory and analgesic activities (Chen et al., 1995). Osthole and xanthotoxin revealed only anti-inflammatory activity, and isoimperatorin only analgesic effect. Interestingly, coumarins can also possess pro-inflammatory effects: lower doses of psoralen and imperatorin have shown an anti-inflammatory effect but at higher doses they have a pro-inflammatory effect (Garcia-argaez et al., 2000). Chronic inflammation induced by cotton pellet granuloma was inhibited by ethanol extracts of Apium graveolens and Ruta graveolens (Atta and Alkofahi, 1998) and R. chalepensis (Alsaid et al., 1990). These extracts showed also an anti-nociceptive effect against both acetic acid-induced writhing and hot plate-induced thermal stimulation in mice indicating central and peripheral effects. Coumarin, or its metabolic products, have the potential to become the treatment of scalds and other forms of thermal wounding because it facilitates the removal of extravasated protein through proteolytic breakdown by stimulated macrophages (Piller, 1997).

Neutrophils play a central role in acute inflammation. Their main function is host protection by generating and releasing agents that destroy invading micro-organisms. Two of the chemoattractants that are involved in the migration of neutrophils from the circulation to the tissue are the phospholipid metabolite platelet activating factor (PAF) and the bacterial product N-formyl-Met-Leu-Phe (fMLP). The functional responses that follow the recognition of these compounds include phagocytosis, secretion of proteolytic enzymes such as elastase, and the production of free oxygen radicals. One approach to measure these functional responses is via PAF induced exocytosis of elastase method developed by Tuominen and co-workers (1992). The reaction of elastase with its substrate leads to formation of a coloured product, which can be quantified by UV measurement. In this work, the effects of twenty coumarins on elastase secretion in human neutrophils were evaluated with the above-mentioned test. Four compounds, namely psoralen, xanthotoxin, ledeboviellol and athamantin, showed significant activity. When the dose-response curves were determined, the potency of athamantin and ledeboviellol was found to be of the same magnitude as that of the reference compound BN 52021, which is a PAF antagonistic gingkolide isolated from the Chinese fossil tree Gingko biloba. None of the four coumarins was selective towards PAF or fMLP receptor because there was no difference between the inhibition against PAF or fMLP stimulated response. One possible target for the compounds may lie in the early phases of intracellular signalling since a common pathway is used in the transduction of signals from both of these receptors. An additional possibility could be interference in calcium regulation of the cell, e.g. inhibition of the receptor-mediated influx of calcium – however, this has not been proven yet. Furthermore, inhibition of the enzyme elastase would give positive results in the assay used. Direct effects on elastase activity were tested and none of the coumarins were identified as elastase inhibitors. Besides the inhibition of neutrophil function studied in this work, coumarin compounds have been found to possess also other anti-inflammatory effects. Due to the low number of compounds found to be active, it is not possible to draw conclusions about the structural features essential for their effect. However, some structural specificity was seen in their action, since some of their structural relatives that were also tested were not capable of inhibiting elastase release: linear furanocoumarins psoralen and 8-methoxypsoralen (xanthotoxin) were active while 5-methoxypsoralen (bergapten) was not; the activity of ledeboviellol disappeared when hydroxyl group was changed to methoxyl group (the 2-methylchromone derivative of ledeboviellol); and angular furanocoumarin athamantin lost its activity when the two iso-valeryl substituents were changed to glucosyl and hydrogen (apterin). Activities of the coumarin compounds in anti-inflammatory tests reported here and in the literature.

Antimicrobial properties

There has been a dramatic increase in pathogen resistance to both pharmaceutical and agrochemical antimicrobial agents. New prototype compounds are needed to address this situation. Successful discovery of novel natural product antimicrobials has necessitated the development of new bioassay techniques and protocols that allow for the detection of small amounts of biologically active chemicals, which should be selective enough to determine optimum target pathogens. Antimicrobial activities have been evaluated with diverse settings often difficult to compare. There are reports on efficacies of pure coumarins against Gram-positive and Gram-negative bacteria as well as fungi, also extracts have shown activities, e.g. methanol extract from Mitracarpus scaber against Staphylococcus aureus and Candida albicans (Bisignano et al., 2000) and water extract from Pelargonium sidosides against Escherichia coli, Klebsiella...
phytoalexins since they are produced by the plant as a defence mechanism against attack by other organisms (Berenbaum, 1991, Weinmann, 1997). The selected plant species had total coumarin contents of between 5 – 834 µg/ml in their methanol leaf extracts calculated from the bergapten standard curve and determined (Vuorela and co-workers 1989), and, as they and the pure coumarins studied were merely active against plant pathogens, The role of coumarins and furanocoumarins as defensive compounds. Extract of P. palustris possessed inhibition against the widest spectrum of plant pathogenic fungi. Extracts of P. crispum and R. graveolens are suggested either to possess good antimicrobial potency or to contain active principle(s), which could belong to the group of coumarins, essential oils or flavonoids. Substances could also be present in the extract that stimulate the growth of the microorganisms, as was especially evident in the case of Phytophthora (cactorum), thus counteracting the effect of inhibitory substances.

Psoriasis is a common skin disease affecting ~2 % of the population (Disepio et al, 1999). While the appearance of the psoriatic skin can vary, each form of psoriasis is characterised by epidermal keratinocyte hyperproliferation, abnormal keratinocyte differentiation and immune-cell infiltration. Psoriasis is often difficult to treat owing to its sporadic course, variable response to treatments and adverse effects (Ashcroft et al, 2000). When searching for suitable therapies for such a complex disease, the effectiveness of the screening method is of utmost importance. Linear furanocoumarin xanthotoxin purified from Ammi majus was first introduced in the treatment of vitiligo over 50 years ago. Investigations of dermatologists led to further development of this therapy for the treatment of psoriasis. Administration of oral or topical psoralens (such as xanthotoxin) followed by irradiation with long wave UV radiation in the 320–400 nm range is now a widely used, frequently convenient and effective systemic treatment of psoriasis with well-characterised and controllable side effects (Lewis et al, 1994; Mcneely and Goa, 1998). PUVA suppresses the accelerated proliferation of the keratinocytes, another mechanism of action in psoriasis is suggested to be a result of its direct lymphotoxic effects. In case of major acute adverse reactions link with PUVA and xanthotoxin (nausea, vomiting, pruritus and erythema), xanthotoxin can be replaced with bergapten. Xanthotoxin may be applied topically as bath before exposure to UVA, advantages of such administration are shorter irradiation times and a lack of gastrointestinal, hepatic or other systemic adverse effects. Trimethylpsoralen (TMP) and its derivatives have been found to inhibit lymphocyte proliferation to a greater extent than xanthotoxin (Berger et al, 1985; Coven et al, 1999), and could provide one of the safest and most effective treatment for psoriasis. Angular furanocoumarins, or angelicins, were long thought to be unable to form crosslinks because of their geometry. The amount of crosslinks formed correlates to the skin-photosensitization. However, it has been shown that 4,6,4’-trimethylangelicin induces crosslinks (Chen et al, 1994; Bordin et al, 1994).

PHOTOTOXICITY
The phototoxic properties of furanocoumarins and related compounds have been assayed using fungi (Gibbs, 1987), green algae (Schimmer et al., 1991), bacteria (Ashwood-Smith et al., 1983), laboratory animals (Nilsson et al., 1993) and cultured human skin systems (Edwards et al., 1994; Damour et al., 1998). The early methods were basically similar to those used for testing the antimicrobial properties of compounds, further coupled with irradiation of the samples by UV light at 366 nm in the presence of the test organism on agar dishes. Later, a thin layer chromatographic procedure was combined with detection of the antimicrobial properties of components in the sample that were separated and nowadays cultured human skin systems are available (Edwards et al., 1994; Damour et al., 1998). Here, a new method employing Artemia salina was developed for phototoxicity testing in order to screen quickly the possibly phototoxic compounds and extracts. In addition, toxicity can be measured at the same time. Artemia salina (brine shrimp) has been successfully used for toxicity testing (Kiviranta et al., 1991; Kiviranta and Abdel-Hameed, 1994). The advantages of A. salina as a test organism are that it is easy to grow from dried eggs viable for several years and easily available, it is small enough to be contained in a small liquid volume, and no complex instrumentation is needed. A. salina larvae are more sensitive to toxicants than adults, it is not necessary to feed them, i.e. to change the saline solution prior or during the test, and they are in a similar physiological condition therefore reducing the variation (van Steertegem and Persoon, 1993). For the development of this method, test compounds were chosen to represent different structural types of coumarins: umbiliferone, a simple coumarin, exhibited no phototoxicity, while the linear furanocoumarins bergapten, psoralen and xanthotoxin showed phototoxic activity, this effect was abolished in the absence of radiation. These results are in accordance with the results of other phototoxicity tests (Ashwood-Smith et al., 1983; Gibbs, 1987; Edwards et al., 1994), and they were the basis for choosing the radiation times for the examination of the plant extracts. Conveniently, A. salina method can be used for simultaneous toxicity studies, and peucedanin was found to be toxic to the larvae irrespective of the irradiation time. Suitability of the A. salina method for the phototoxicity testing of plant material was tested with leaf extracts of six plants from Apiaceae and one from Rutaceae. Leaf extracts containing one or more above mentioned linear coumarins exhibited phototoxic activity with the exception of the extract of Anethum graveolens, which was poor in coumarin content. Leaf extracts of Levisticum did not contain any of these three linear coumarins, and neither toxic nor phototoxic effects were observed, while Ruta graveolens exhibited both phototoxicity and toxicity. In order to investigate the influence of the changes in total coumarin content during the growth period, A. salina tests were performed at different stages of flowering. Aegopodium podagraria showed the most remarkable changes: the coumarin content decreased but toxic effects appeared, which indicated an increase of some other compounds responsible for this phenomenon. With the method described here it is not possible to distinguish active and inactive components in a mixture or in an extract. However, the overall procedure is rapid: one can screen active plant extracts, isolate compounds from them for structural elucidation, and finally screen the compounds for activity. A. salina screening test supplement animal bioassays with which there can be problems caused by differences for example in skin penetration and the presence of hair. Active compounds verified by the A. salina screening test could later be subjected to more elaborate bioassays like laboratory-grown human dermis and epidermis.

Effects on calcium fluxes

The entry of calcium into the cell occurs through various channels: e.g. voltage operated calcium channels (VOCCs), receptor operated channels, and calcium release activated channels (Castaldo and Capasso, 1996). So far, six types of VOCCs (N, T, L, P, Q, R) have been identified (Alexander and Peters, 1998; Denyer et al., 1998). The channels are transmembrane proteins with an ion-selective aqueous pore that, when open, extends across the membrane (Denyer et al., 1998). Channel opening and closing (gating) is controlled by a voltage-sensitive region of the protein containing charged amino acids that move within the electric field. The movement of these charged groups leads to conformational changes in the structure of the channel resulting in conducting (open/activated) or nonconducting (inactivated) states. Depolarisation, ligands and mechanical factors control the calcium influx by regulating how long the calcium channel is open (Nayler, 1993). A working model of the modulation of (Ca$^{2+}$)i in GH4C1 cells (Wagner et al., 1993). In general, inositol phospholipids are broken down by phosphoinositide specific phospholipase C (PLC) in response to many agonists, e.g. thyrotropin-releasing hormone (TRH) or ATP, (Wagner et al., 1993; Berridge, 1995; Berridge et al., 2000). The generated products, inositol-1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG), serve as second messengers and play a role in intracellular Ca$^{2+}$ mobilisation and in the activation of protein kinase C, respectively. At normal physiological stimulation levels IP3 may increase the sensitivity of the IP3 receptor to Ca$^{2+}$, resulting in a process called Ca$^{2+}$ induced Ca$^{2+}$ release (CICR). In most cells, stimulation with agonists leads to emptying the Ca$^{2+}$ stores, which in turn activates the storeoperated Ca$^{2+}$ channels (SOCCs) leading to Ca$^{2+}$ entry through an unknown mechanism. A conformational-coupling mechanism has been suggested, which proposes that IP3 receptors in the endoplasmic reticulum are directly coupled to SOCCs. Once Ca$^{2+}$ has carried out its signaling functions, it is rapidly removed from the cytoplasm by various pumps and exchangers. In rat thyroid FRTL-5 cells, the regulation of Ca$^{2+}$ entry occurs via receptor- and store-operated pathways (Törnquist, 1992, 1993). GH4C1 cells contain at least two functionally distinct intracellular Ca$^{2+}$ stores (Wagner et al., 1993). The first store is IP3-sensitive and releases Ca$^{2+}$ in response to TRH. This store is sensitive to emptying by thapsigargin, but maintains its ability to respond to TRH in the absence of extracellular Ca$^{2+}$. The second pool is not sensitive to emptying by thapsigargin. Antagonists like nifedipine decrease the fluctuations in intracellular concentration of free calcium ((Ca$^{2+}$)i) by inhibiting Ca$^{2+}$ influx through L-type VOCCs.
GH4C1 cells are able to decrease \((\text{Ca}^{2+})_i\) by at least three different mechanisms: \(\text{Ca}^{2+}\)-uptake into intracellular stores, \(\text{Ca}^{2+}\)-efflux via \(\text{Na}^+ / \text{Ca}^{2+}\) exchanger, and \(\text{Ca}^{2+}\)-efflux via plasma membrane \(\text{Ca}^{2+}\)-ATPase (Wagner et al., 1993). There are many plant-derived compounds active on calcium channels, which have been extensively reviewed (Vuorela et al., 1997). Coumarins have a possible calcium blocking activity studied mostly with vascular preparates, e.g. dihydropyranocoumarin visnadin isolated from \textit{Ammi visnaga} fruits (Rauwald et al., 1994a; Duarte et al., 1997), 6,7-dimethoxycoumarin scoparone isolated from \textit{Artemisia capillaris} (Yamahara et al., 1989), columbianadin isolated from \textit{Peucedanum palustre} (Törnquist and Vuorela, 1990; Vuorela, 1988), ostruthol isolated from \textit{Peucedanum ostruthium} (Rauwald et al., 1994b), osthol isolated from \textit{Angelica archangelica} (Härmälä et al., 1992). Aqueous extract of common rue (\textit{Ruta sp.}) has shown positive chronotropic and inotropic effects.
effects on isolated right atria of normotensive rats (Chiu and Fung, 1997). It also relaxed KCl precontracted rat tail artery strips probably by a direct effect on the vascular smooth muscle. Extract from *R. graveolens* proved to, besides K⁺-currents, also block Na⁺-currents, although to a lesser extent, in intact myelinated nerve fibres (Bethge et al, 1991). The interference at different levels of the cellular calcium regulation demonstrates that many of natural calcium antagonists represent a promising field of research for identifying derivatives which are more effective or able to react on structures not sensitive to synthetic calcium antagonists (Castaldo & Capasso, 1996). It has been suggested that these drugs could be useful tools to better understand channel kinetics and calcium mobilisation from intracellular deposits and to proceed to the synthesis of new molecules with calcium antagonistic action.

Columbianadin, isolated from *Peucedanum palustrum* roots, has been shown to possess calcium channel blocking activity in the test with rabbit aortic rings, the test system measuring calcium-dependent prolactin release with rat pituitary GH3 cells, and depolarisation-induced uptake of calcium with a potency comparable to verapamil (Vuorela et al, 1988b; Törnquist and Vuorela, 1990). Athamantin, isolated from *P. oроselinum*, showed the highest activity in inhibition of depolarisation induced uptake of 45Ca²⁺ in rat pituitary GH4C1 cells when athamantin and four linear coumarins were tested (Hadácêk et al, 1991). Archangelicin, osthol, isoimperatorin, imperatorin and phellopterin, all isolated from *Angelica archangelica* roots, inhibited the uptake of 45Ca²⁺ in GH4C1 cells (Härmlä, 1991). These results lead to further investigations presented in this thesis, and a simple coumarin, osthol, a linear furanocoumarin, xanthonotoxin, and an angular furanocoumarin, columbianadin, were chosen to represent different structures of coumarins.

FRTL-5 rat thyroid cells were chosen as the test system because they are non-excitabil cells with both receptor- and store-operated calcium entry pathways (Törnquist, 1992, 1993). The rationale for using GH4C1 cells is that the calcium channels in these cells have been thoroughly characterised and the cells used in a multitude of studies on calcium channels (Koshiyama and Tashjian, 1991; Törnquist, 1991). In rat thyroid FRTL-5 cells, osthol prevented the ATP-induced influx of extracellular calcium. The effect of osthol on the ATP-evoked increase in (Ca²⁺)i was probably due to modification of the IP3 pathway, because the calcium transient was decreased in medium containing calcium and medium without calcium. Osthol also decreased the thapsigargin evoked change in (Ca²⁺)i in buffer containing calcium, suggesting that osthol inhibited the thapsigargin evoked influx of extracellular calcium (*i.e.* store-operated calcium entry). Furthermore, part of the decreased ATP response was probably also due to an attenuation of store-operated calcium entry, as the thapsigargin evoked increase in (Ca²⁺)i also was attenuated. Columbianadin had no effect on the ATP-evoked increase in (Ca²⁺)i. The mechanism of action of osthol and xanthotoxin on agonist-evoked changes in (Ca²⁺)i were further delineated in rat pituitary GH4C1 cells. Especially the effects of these compounds on the ligand-receptor interaction were possible to test as the binding of TRH to its receptors is well characterised and relatively easy to measure (Hinkle, 1989; Gershengorn and Osman, 1996). The results show that osthol, but not xanthotoxin, interferes with the binding of TRH to its receptor, and thus alters receptor-evoked intracellular signals, *i.e.* the production of IPs and the release of sequestered calcium. The mechanism through which osthol interacted with the receptor is not clear at present. It may function as a competitive antagonist, or it could modulate the binding indirectly, *i.e.* by modulating receptor-G protein interactions, or it may activate intracellular signalling pathways which could modulate receptor function. However, it is clear that the effect on the receptor is rather compound-specific than a nonselective feature of all coumarins.

**Other biological effects and toxicity**

Linear furanocoumarin xanthonotoxin is capable of inactivating human P450 2A6, the major coumarin 7-hydroxylase present in human liver, at physiologically relevant concentrations (Koenigs et al, 1997) and bergapten against intestinal CYP3A4 (Ho et al, 2000), and therefore these compounds carry the potential of causing a serious drug-drug interaction with any drug, compound or toxin whose clearance is largely dependent on these enzymes. Psoralen, xanthonotoxin and spiondrol proved to be inhibitors of coumarin 7-hydroxylase activity both in mice and in human liver microsomes (Mäenpää et al, 1993). Woo and co-workers (1983) investigated the effects of coumarins from *Angelica koreana* on the drug-metabolising enzymes and found imperatorin, isoimperatorin, oxypeucedanin, isoxyypeucedanin, and oxypeucedanin methanolate to retard the drug metabolism. Praeruptorin A, xanthonotoxin, psoralen and bergapten isolated from chloroform extract of the root of *Peucedanum japonicum* inhibited monoamine oxidase (mouse brain) (Huong et al, 1999), and daphnetin proved to be a protein kinase inhibitor in human hepatocellular carcinoma HepG2 cells (Yang et al, 1999). In sensitive tumour cells, coumarin and its derivatives cause significant changes in the regulation of immune responses, cell growth and differentiation (Seliger, 1997). Coumarins appear to act either directly on tumour cells, or via modulation of the host’s immune system, thereby stimulating immune reactivity which leads to protection against recurrence of a particular tumour or even to activation of host defence mechanisms which also help to eliminate small tumour burdens (Zlabinger, 1997). Direct (*i.e.* non-immunologically mediated) antitumor effects have been shown in a number of studies demonstrating a growth inhibitory capacity for a number of malignant cell lines in *vitro*, *e.g.* extract of the root of *Angelica japonica* (containing scopoletin, japoangelone, oxypeucedanin methanolate, xanthotoxin, bergapten) against human gastric adenocarcinoma MK-1 cell growth (Fujikota et al, 1999), methanol extract of *Tordyllum apulum* (containing umbelliferone, isooimperatorin and an angelicin derivative) against the KB (human rhinopharynx cancer) and NSCLC-N6 (human bronchial epidermoid carcinoma) cancer cell lines (Kofinas et al, 1998). The toxicological profile of coumarin has been somewhat ambiguous, therefore the coumarin for toxicity and carcinogenicity studies (Weinmann, 1997). The report states that organ-specific toxicity
occurs in species and strains only, that metabolise coumarin qualitatively and quantitatively different from man, and in rodents, chronic lesions and tumorigenesis might be seen after overdosing of the compound for months to years. According to Lake (1999), the majority of tests for mutagenic and genotoxic potential suggest that coumarin is not a genotoxic agent, and exposure to coumarin from food and/or cosmetic products poses no health risk to humans. However, the possibility for phototoxic effects of furanoderivatives of coumarin as well as hepatotoxic aflatoxins, metabolites from Aspergillus species, should be born in mind.

USE OF COUMARINS IN PHARMACEUTICAL AND CHEMICAL INDUSTRY

The bioactivities of phototoxic psoralens and of dicoumarol derivatives are well known and several of these compounds are used in antipsoriatic and anticoagulant therapy, respectively (Honigsmann et al, 1989; Matern et al, 1999). Besides psoriasis, skin diseases like cutaneous T-cell lymphoma, atopic dermatitis, alopecia areata, urticaria pigmentosa and lichen planus (Oliver and Winkelmann, 1993; Goodman and Gilman, 1996) are treated with the photochemotherapy with linear furanocoumarins (also referred to as psorales) and UVA. The most widely used compound is xanthotoxin (Conconi et al, 1998). Bergapten is considered a valuable alternative for chemotherapy of psoriasis, since its clinical efficacy is comparable to that of xanthotoxin, although bergapten requires significantly higher cumulative UVA doses. Since skin phototoxicity and genotoxicity seem to be related to the formation of diadducts to DNA, several monofunctional compounds have been synthesised. The introduction of methyl groups at positions 3 or 4 and 4’ of the tricyclic structure of xanthotoxin led to compounds such as 3,4’-dimethyl xanthotoxin, entirely monofunctional, which is not genotoxic and phototoxic, although it shows an elevated antiproliferative activity. These features also appeared in methylangelicins. Coumarin is the parent molecule of warfarin, which acts as a vitamin K antagonist. Warfarin is a clinically useful anticoagulant and widely employed rodenticide whose discovery was based on the studies of the bleedings of cows suffering from ‘sweet clover disease’ (sweet clover = Melilotus officinalis) (Hoult and Payá, 1996). In the treatment of small-cell lung cancer, the use of warfarin, in conjunction with standard chemotherapy, produces a higher response rate than chemotherapy alone (Zacharski, 1994). The usefulness of coumarins and coumarin derivatives has been shown in various areas of analysis (Cooke et al, 1997). The inherent fluorescent properties of many coumarins are a key factor in many applications. Areas where coumarins are widely used include estimation of enzymatic activity (derivatives of 7-hydroxycoumarin as fluorogenic enzyme substrates; Egan et al, 1990), labelling of proteins, antibodies, DNA and lipids (aminomethyl coumarin acetic acid fluorescent labelling antibodies and lectins for staining), derivatising agents in chromatography, dyes for tuning lasers in ion analysis (7-amino-4-methylcoumarin, 4-methylumbelliferone), intracellular ion indicators, pH and gas detection, measurement of drug/ion transport, studies on bioreactor characterisation, chemical markers in kerosene, food adulterant detection and in sensors. 3,4-Dichloroisocoumarin is a commercially available, relatively non-toxic inhibitor, which shows good reactivity with a large number of serine proteases (Beynon & Bond, 1989). Coumarin has a wide variety of uses in industry, mainly due to its strong fragrant odour (Egan et al, 1990). Its uses include that of a sweetener and fixative of perfumes (3,4-dihydrocoumarin), an enhancer of natural oils, such as lavender, a food additive in combination with vanillin, a flavour/odour stabiliser in tobacco, an odour masker in paints and rubbers, and, finally, it is used in electroplating to reduce the porosity and increase the brightness of various deposits, such as nickel. 6-methylcoumarin is mainly used as a flavour enhancer, and 7-hydroxycoumarin in sunscreens.

DISCUSSION

Natural coumarins, like other unsaturated lactones, may exert various effects on living organisms. The extensive range of physiological effects, both in plants and in animals, in addition to low toxicity, relative cheapness, presence in the daily groceries as spices, fruit and vegetables and occurrence in various herbal remedies of coumarins, led us to investigate more thoroughly properties of some natural coumarins and coumarin containing extracts with ethnopharmaceutical background. Twenty coumarin compounds were studied for their anti-inflammatory properties in an in vitro model for elastase secretion in human neutrophils using PAF and FMLP as stimuli. The activities of the compounds used were relatively modest and non-specific, psoralen, xanthotoxin, ledebouviellol and athamantin being the most active ones. The anti-inflammatory activity may be involved in their ethnobotanical use as remedies for cough, cold and arthritis. Besides anti-inflammatory activity, psoralen and xanthotoxin were active also in the photoxicity tests, and both of these activities may support their use in the treatment of psoriasis. Antimicrobial screening against selected Gram-positive and Gram-negative bacteria, yeasts, mold, as well as plant pathogenic fungi, with emphasis on method optimisation was carried out on methanol extracts prepared from seven plants growing in Finland. The selected plant species were merely active against plant pathogens which support the role of coumarins and furanocoumarins as defensive compounds rather for the plant itself. Linear furanocoumarins bergapten, psoralen and xanthotoxin were phototoxic and umbelliferone as a simple coumarin was not, as expected along with earlier observations. Methanol extracts from seven coumarin containing plants were also studied, and the clearest phototoxic activity was observed with Angelica archangelica throughout the vegetative period and with Peucedanum palustre at later stages of growth. Additionally, it is possible to investigate also toxicity at the same time with the same concentrations, as seen with psuedanin and the extract of Ruta graveolens. Based on earlier observations (Törnquist and Vuorela, 1990; Häräälä et al, 1992), cumbrianianin, osthol and xanthotoxin were used to evaluate further the mode of action on calcium fluxes in rat thyroid FRTL-5 cells as well as in rat pituitary GH4C1 cells.
CONCLUSIONS

The results suggest that osthol may prove to be a useful compound for investigations on ligandreceptor interaction, and some coumarins may have a dual mechanism of action: in addition to their earlier documented effects on VOCCs, they may also interact with receptor-mediated signaling events. In conclusion, coumarin compounds can be suggested to be beneficial for the plants themselves as natural biocontrolling antipathogenic compounds, and for human beings as dietary supplements on the basis of their mild antimicrobial and anti-inflammatory effects, and as reference compounds in various bioactivity tests. The use of these compounds as medicinal agents is of importance in the case of hyperproliferative skin diseases like psoriasis.

REFERENCES


Cotton, C.M., 1996. Ethnobotany-principles and applications, John Wiley & Sons Ltd, Chichester, UK.


Goodman, A., Gilman, A., 1996. The pharmacological basis of therapeutics, chapter 64 dermatological pharmacology. 9th ed. The mcgraw-hill companies, USA.


Hälvä, S., 1988. Culinary herbs and spices of finland. in: craker, L.e. and simon, j.e. (eds.) herbs related to traditional medicine, parthenon europe ltd, uk.


Rauwald, H.W., Brehm, O., Odenthal, K.P., 1994b. Screening of nine vasoactive medicinal plants for their possible calcium antagonistic activity. strategy of selection and isolation for the active principles of olea europaea and peucedanum ostruthium. Phytotherapy Research 8, 135-140.


