REFERENCES

Angelica archangelica
ANGELICA

1. **Newall**, Carol A.; **Anderson**, Linda A. and **Phillipson**, J. David: Herbal Medicines - a guide for health-care professionals. London. The Pharmaceutical Press. 1996. ISBN No. 0-85369-289-0.

Angelica archangelica
ANGELICA

Species (Family)
Angelica archangelica L. (Apiaceae/Umbelliferae)

Synonyms(s)
Archangelica officinalis Moench. or Hoffm.

Part(s) Used
Fruit, leaf, rhizome, root

Pharmacopoeial Monographs
BHP 1983
BPC 1934
Martindale 28th edition

Legal Category (Licensed Products)
GSL

Constituents
Coumarins Angelicin, osthol (major constituent in rhizome/root at 0.2%), bergapten, imperatorin (major constituents in fruit at 0.1% and 0.5% respectively), oreoselone, oxypeucedanin, umbelliferone, xanthotoxin, xanthotoxol.
Volatile oils (0.3 - 1.0%, highest in fruit) Major components include α- and β- phellandrene, α-pinene, α-thujene, limonene, β-carophyllene, linalool, borneol, acetaldehyde, and four macrocyclic lactones.
Other constituents Archangelenone (a flavonoid), palmitic acid, sugars (fructose, glucose, sucrose, umbelliferose).

Food Use
Angelica is widely used in foods. The related species *Angelica silvestris* is listed by the Council of Europe as a source of natural food flavouring (category N3). This category indicates that A *silvestris* can be added to foodstuffs in the traditionally accepted manner, although there is insufficient information available for an adequate assessment of potential toxicity. In the USA, angelica is listed as GRAS (generally regarded as safe).

Herbal Use
Angelica is stated to possess antispasmodic, diaphoretic, expectorant, bitter aromatic, carminative, diuretic, and local antiinflammatory properties. It has been used for respiratory catarrh, psychogenic asthma, flatulent dyspepsia, anorexia nervosa, rheumatic diseases,

peripheral vascular disease and specifically for pleurisy and bronchitis, applied as a compress, and for bronchitis associated with vascular deficiency.

Dose
Dried leaf: 2 - 5 g or by infusion three times daily
Leaf liquid extract: (1:1 in 25% alcohol) 2 - 5 ml three times daily
Leaf tincture: (1:5 in 45% alcohol) 2 0 5 ml three times daily
Dried rhizome/root: 1 - 2 g or by infusion three times daily
Rhizome/root liquid extract: (1:1 in 25% alcohol) 0.5 - 2.0 ml three times daily
Rhizome/root tincture: (1:5 in 50% alcohol) 0.5 - 2.0 ml three times daily
Fruit: 1 - 2 g.

Pharmacological Actions
Animal studies Minimal anti-inflammatory activity (1% inhibition of carrageenan-induced rat paw oedema) has been documented for fruit extract (100 mg/kg body-weight by mouth) given 45 minutes before eliciting oedema. This was compared to 45% inhibition by indomethacin (5 mg/kg by mouth). Angelica is reported to possess antibacterial and antifungal properties. Antibacterial activity against *Mycobactrium avium* has been documented, with no activity exhibited against *Escherichia coli, Bacillus subtilis, Streptococcus faecalis,* or *Salmonella typhi*. Antifungal activity was reported in 14 of 15 fungi tested.

Furanocoumarins isolated from a related Chinese species, *Angelica koreana* have been reported to affect the hepatic metabolism of hexobarbitone. The compounds were found to cause a marked inhibition of drug metabolism in the first phase and an acceleration in the second phase, and were thought to be drug-metabolising enzyme inhibitors rather than enzyme inducers. Furanocoumarins investigated included imperatorin and oxypeucedanin, which are also documented as constituents of *A. angelica*. It has been reported that a related Chinese species, *Angelica sinensis*, may be hepatoprotective and prevent the reduction of hepatic glycogen.

In the rabbit, a uterotonic action has been documented for Japanese angelica root following intraduodenal administration of a methanolic extract (3 g/kg). *A. sinensis* is reported to have induced uterine contraction and relaxation.

Human studies None documented for angelica (*A. archangelica*). Many related species are traditionally used in Chinese medicine. *A sinensis* has been reported to be effective in improving abnormal protein metabolism in patients with chronic hepatitis or hepatic cirrhosis.

The furanocoumarin constituent bergapten (5-methoxypsoralen) has been used in the PUVA treatment of psoriasis.

Side-effects, Toxicity
Both angelica and the root oil have been reported to cause photodermatitis and phototoxicity, respectively, following external contact. Angelica contains furanocoumarin constituents which are known to cause photosensitisation. Concern has been expressed at the possible carcinogenic risk of the furanocoumarin bergapten.

The root oil has been reported to be non-irritant and non-sensitising on animal and human skins.

Root and fruit oils obtained by steam distillation are claimed to be devoid of furanocoumarins, although extracts may contain them.
Toxicity studies have been documented for the root oil. Acute LD_{50} values have been reported as 2.2 g/kg body-weight (mouse, by mouth), and 11.16 g/kg (rat, by mouth). Death was attributed to liver and kidney damage, although animals surviving for three days completely recovered with a reversal of organ damage. An acute LD_{50} (rabbit, dermal) value was reported to be greater than 5g/kg. Subacute toxicity studies lasting eight weeks, suggested that the tolerance dose in the rate was 1.5 g/kg, although at lower doses the animals weighed less than the controls.

Contra-indications, Warnings
Angelica may provoke a photosensitive because of the furanocoumarin constituents. Excessive doses may interfere with anticoagulant therapy, because of the coumarin constituents.

The use of bergapten in cosmetic and suntan preparations is stated to be ill-advised by some regulatory authorities in view of the concerns regarding the risk of cancer. The International Fragrance Association recommends that Angelica root oil be limited to a maximum of 0.78% in products applied to skin which is then exposed to sunshine.

Pregnancy and lactation Angelica root is reputed to be an abortifacient and to affect the menstrual cycle. In view of this and the photosensitising constituents, the use of angelica during pregnancy and lactation in amounts exceeding those used in foods should be avoided.

Pharmaceutical Comment
The chemistry of angelica is well documented. Although the traditional use of Chinese angelica species, such as *A. sinensis* and *A. acutiloba* is well established in oriental medicine, there is limited documented pharmacological information available for *A. archangelica*, the species commonly used in Europe, to justify its herbal uses. In view of the presence of known pharmacologically active constituents, especially bergapten, consumption of amounts exceeding normal human dietary intake should be avoided. Angelica contains furanocoumarins which are known to possess photosensitising properties.

2. Back in her book (B55) reports that Angelica has an interesting folk lore. In the Middle Ages when Europe was ravaged by the Plague, an angel apparently came to a monk in a vision, telling himn that this herb would effect a cure - and ever since then it has borne the name Angelica.

It is a good remedy for indigestion and flatulence. It ius a tonic herb, stimulating the appetite. It has a useful role in feverish colds. Can be used for coughs and sore throats when used as the dried roots. It can be used as a mouthwash to sweeten the breath.

In beauty care it can be used to soothe the skin and to stop itching, a fresh compress can be made of Angelica leaves, or use Angelica ointment made from fresh or dried roots. The delicate fragrance of angelica adds a soothing freshness to the skin when added to the bath water.

3. Ceres in their book (B2) refer to it as the Angels' Herb or Garden Angelica. It grows wild throughout Europe and at higher elevations in the western and central United States. Angelica is used by the herbalists as a digestive (carminative) herb, for those with wind in the stomach, also

as a tonic and an expectorant and stimulative drink. IT MUST NOT BE TAKEN BY DIABETICS, AS IT ENCOURAGES THE PRODUCTION OF TOO MUCH SUGAR IN THE BODY.

4. Fluck in his book (B1) says that European or Garden Angelica contains volatile oil and derivatives of coumarin. It stimulates the digestive secretions, increases appetite and controls peristalsis. In large doses it stimulates then paralyses the central nervous system. It is a principal additive of Chartreuse and Benedictine liqueurs.

5A. Leyel in her book (B9) says that the great reputation of Angelica in ancient times is shown by its botanical name today. It is little used as a medicine and now mainly used as a flavouring. Helps produce perspiration in feverish colds when used in combination with other herbs. Its flowering coincides with Archangel Michael's own day, 8th. May. The Letts endowed Angelica with magical powers, and the songs which are chanted by them when the herb is carried to market are very ancient. The Laplanders believe that it prolongs life, and they chew it and smoke it in the same way as tobacco.

Angelica is cultivated in a large scale in France - near Clermont Ferrand -and is sold for making liqueurs and sweets. It is said to be in Chartreuse, anisette and of French Vermouths. Aids digestion, seeds used in medicine, though some prefer the root. The Norwegians and Laplanders use the root for bread. The plant has the reputation of causing a distaste for alcohol in those who drink too much.

5B. Hoffmann reports in his book (B7) that the roots and leaves are used medicinally. It contains essential oils including phellandrene and pinene, angelica acid, coumarin compounds, bitter principle and tannin. It is a carminative, antispasmodic, expectorant, diuretic and diaphoretic. Useful expectorant for coughs, bronchitis and pleurisy, especially when they are accompanied by fever, colds and influenza. The leaf can be used as a compress in inflammations of the chest. Eases intestinal colic and flatulence. Stimulates the appetite and may be used in anorexia nervosa. It has been shown to help ease rheumatic inflammations.

6. Lautier and Passebecq (B18) say that Angelica contains Phellandrenic compounds, organic acids, angelicin (a coumarin). Bergaptene. Imperatorin (two furanocoumarins).

It is used in anorexia, dyspepsia, stomach ulcers. In the form of an ointment it has a soothing effect on skin complaints, arthritis and rheumatism. It is used in thecomposition of stomachics.

7. Potter in his book (B5) tells us that Angelica is known as an aromatic, stimulant, carminative, diuretic and diaphoretic. It was included in the 1934 British Pharmaceutical Codex in Warburg's Tincture but omitted from the next edition. It was described as an antispasmodic.

8. Weiss (B46) says that there is a wild Angelica called Angelica sylvestris which is very similar in appearance, it grows in damp grassy places, in woods and on cliffs, but lacks the penetrating aromatic fragrance of the garden Angelica. Angelica contains bitters and 0.35-1.0% of volatile oil, as well as lactone angelicin, resins, tannins, and 0.3% of angelic acid - all in all quite a complex composition. Used in liqueurs as already mentioned previously. Has carminative properties, counteracts flatulence, applied externally for rheumatism (contains camphor and alcohol).

9. Grieve has a large section on Angelica (B6) and says that there are about thirty varieties but

that this is the only one used medicinally. Its virtues are praised by the old writers and the name itself testifies to the great antiquity of a belief in its merits as a protection against contagion, for purifying the blood and for curing every conceivable malady. Also called the root of the Holy Ghost. Grieve gives a great deal more history. The herb is used in sweets and liqueurs as already discussed. Angelica has carminative, stimulant, diaphoretic, stomachic, tonic and expectorant properties, which are strongest in the fruit, thought the whole plant has these virtues. Good for colds, coughs, pleurisy, wind, colic, rheumatism and diseases of the urinary passage. IT SHOULD NOT BE GIVEN TO DIABETICS, AS IT CAUSES AN INCREASE OF SUGAR IN THE URINE.

It is a useful agent for feverish conditions as a diaphoretic. Used as a poultice externally for lung and chest disease. Juice from stem is good for chronic rheumatism and gout. Taken in medicinal form it is said to cause a disgust for spiritous liquors!
Norwegians make bread from the roots. The stems can be used as traps for earwigs!

10. Lust in his book (B8) refers to three varieties of Angelica namely:-Angelica archangelica - European Angelica or Garden Angelica Angelica sylvestris - Wild Angelica , European Wild Angelica or goutweed Angelica atropurpurea - American Angelica, high Angelica, belly ache root, archangel, purple Angelica ,etc.

The Garden Angelica is the only one that will be discussed here. Appetiser, carminative, expectorant, stimulant, stomachic, tonic. The seeds are said to be diaphoretic and diutetic. Good for flatulence, colic, ulcers, vomiting, stomach cramps. Also for intermittent fever, nervous headache, and general weakness. A decoction of the root can also be used for scabies or itching and also for wounds. As a compress in gout.

11. In a book by Schauenberg and Paris (B60) we read that angelica contains essential oil. The root contains 1% of this, consisting of phellandrene, organic acids, and a coumarin (angelicin). The seeds contain furanocoumarins (imperatorin and bergaptene). Angelica is tonic, carminative, stomachic and antispasmodic. The rhizome produces angelica oil which is a restorative. It can be used externally to soothe rheumatism, arthritis and skin disorders. Also used internally for anorexia, dyspepsia, stomach ulcers and as an expectorant.

12. In a reference from Manufacturing Chemist Februaury 1991. p.43. We learn that a European Patent application 0 357 081 (7.3.90) by Kao Corporation describes a bathing preparation that is said to have a warming effect and accelerate blood circulation. It comprises an alkyl phthalide and/or an alkoxy phthalide; carbon dioxide; or a material capable of generating carbon dioxide; together with a plant belonging to the family umbelliferae. Some herbs are believed to have a warmth generating effect when they are included in bath preparations, and many such products cuurently on the market contain fine pieces of herb or herb extracts. Herbs, rhizomes or the roots of plant belonging to the Umbelliferae family, such as Angelicae radix and Cridium rhizoma are said to have excellent effects. However, to be effective these herbs may have to be used in large ammounts, which may give turbid bath water. One solution might be to use extracts, but this is uneconomical, does not cure the turbidity, and the extraction reduces the bathing effects. The odour may not be pleasant. The special synthesis is the subject of the patent.

13.Buchman says that angelica tea is useful for delayed menstrual period and for overcoming the effects of stomach gas. Hemlock flowers look similar to wild angelica, be sure to know the difference.

14. In a data sheet from Ransom we read that in skin care it stops itching and is soothing. It gives a relaxing bath and makes a foot bath for tired feet. It is used as an eye wash for sore eyes. Medicinally it is diaphoretic and expectorant. The seeds are burnt as a room freshener. It is a herb of the sun in Leo.

15. Levy (B77) says that it is *Archangelica officinalis* which has umbrels of white flowers and a most powerful and fragrant scent. The leaves, broad leaf stalks and roots are used, especially stalks. Also the seeds. It is good for digestive troubles, heartburn and colic. In France and Spain the candied plant is valued for its tonic properties and for fertility. The tea is a good eye tonic.

16. In a data sheet from Maruzen Pharmaceuticals (through K&K Greeff) we read that the roots of *Angelica dahurica* are used to produce an extract that is skin regenerating and antibacterial.

In another reference from this document we read that the roots of *Angelica acutiloba* or Japanese Angelica are used to prepare an extract that is antiphlogistic and helps regenerate the skin.

17. In a data sheet from Maruzen Pharmaceuticals (through K&K Greeff) we read that Japanese Angelica or *Angelica acutiloba* is widely formulated in many Chinese indigenous medicines including a simotuto, touki syakuyakusan and toukisigyakutou and is an important drug for gynaecological diseases such as menoxenia, menorrhalgia, blots and freckles, frostbite and chilly sensations.

As the medical effects of Japanese Angelica root are well known, namely, analgesic, anti-inflammatory and blodd complimenting action. Additionally, it gives a fair complexion against blots and is used as a material for skin care products.

Fair complexion effect, prevention of sunburn, cell activation action, sweating action and promotion of blood circulation.

There is an ingredient which inhibits tyrosinase activity and to protect against UV rays. It is also combined in bath medicines and is an ingredient of essential oils - ligustilide - which promotes blood circulation and prevents the chilly sensation felt after bathing.

The extract demonstrated tyrosinase inhibition of 16%, 21% and 52% at concentrations of 2%, 5% and 10% respectively.

Contains phthalides (ligustilide), coumarins (dergapten), vitamin B12 and nicotinic acid.

18. Reid (B191) refers to *Angelica anomola* (Umbelliferae) as Bai Zhi. It is found in China, Japan, where the roots are used.
Nature: Pungent and bitter; warm.
Affinity: Lungs, stomach
Effects: Analgesic in wind-injury; reduces swelling; antidote.
Indications: Colds, headaches, aches and pains due to wind-injury; abscesses and swelling; leucorrhoea; congestion; snake-bites.
Dosage 4-7g.
Remarks: Important ingredient in antidote potions for poisonous snake-bited.

19. Hirano, M; Matsumoto, T; Kiyohara, H; Yamada, H.: Lipopolysaccharide-independent limulus amebocyte lysate activating, mitogenic and anti-complementary activities of pectic polysaccharides from Chinese herbs. Planta Medica (1994) 60(3): 248-252. [En, 20 ref.] [Oriental Medicine Research Center of the Kitasato Institute, Tokyo 108, Japan.]

A crude pectin fraction (BR-2) and 2 pectin-like substances (bupleuran 2IIb and 2IIc), isolated from the roots of Bupleurum falcatum, a pectic polysaccharide (GR-2IIc), isolated from the roots of Glycyrrhiza uralensis, and a pectic arabinogalactan (AGIIb-1), isolated from the roots of Angelica acutiloba, were tested for the presence of lipopolysaccharide (LPS) or LPS-like substances, using the chromogenic Limulus amoebocyte lysate (LAL)-assay. Each compound responded positively to Toxicolor and Endospecy (LAL-test kits containing factors C+G and C, respectively), with AGIIb-1 showing the highest reactivity rates. The fractions GR-211c/PG-1 (ramified region; rhamnogalacturonan core with neutral sugar side chains) and AGIIb-1 were found to activate the endotoxin-mediated coagulation factor (factor C). When bupleuran 2IIc was digested with endo-polygalacturonase or degraded with lithium, the resulting oligosaccharides still showed LAL-reactivity. Carboxyl-reduction of acidic polysaccharides increased the reactivity to the beta-(1->3)-glucan-mediated coagulation factor (factor G) but not to factor C. Bupleuran 2IIc and its PG-1 and PG-2 (rhamnogalacturonan II-like region, which contains 2-keto-3-deoxyoctulosonic acid) showed significant mitogenic activities in mice. When the LAL-positive substance was removed from bupleuran 2IIc and its fraction PG-1 by phase separation using Triton X-114, the in vitro mitogenic and anticomplementary activities did not change. It is concluded that the pectic polysaccharides studied contained LPS or LPS-like substances but that their anticomplementary and mitogenic activities were not due to the action of LPS substances.

KEYWORDS: *Angelica acutiloba*; Apiaceae; *Bupleurum falcatum*; complement; *Glycyrrhiza uralensis*; immunological properties; lipopolysaccharides; mitogens; oligosaccharides; Papilionoideae; pharmacology; polysaccharides; roots

20. THE LAWRENCE REVIEW OF NATURAL PRODUCTS
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ANGELICA

Scientific name: *Angelica* spp. Family: Umbelliferae

Common names: Angelica, wild angelica, garden angelica

Botany: Angelica is a tall, aromatic biennial plant of the parsley family. It possesses deeply

indented, very large leaves and strong stems. The plant is commonly used as an attractive border for herb gardens and to shield other herbs from the wind. the stems, leaves and flowers are light green in colour. The species *A. archangelica*, also referred to as *A. officinalis* (Moench.) Hoffm., is native to shady places in Iceland, Lapland and other northern regions. The species *A. atropurpurea* is found in North America, and *A. sylvestris L.* is a small European species. Other species include *A. curtisi* and *A. rosaefolia. A. atropurpurea* is also known in the United States by the common name alexanders, but this name is also used to identify another related plant, *Smyrnium olusatrum. A. pubescens* roots are used in Chinese herbal medicine for the treatment of arthritis, headaches and as a carminative.

History

According to legend, angelica was revealed to humans by an angel as a cure for the plague, hence its name. It was introduced to England during the 16th century. Angelica is best known today in the form of candied or crystallised stems. Dried leaves have been used to make tisanes, which resemble Chinese tea , and as a scent in potpourri. Angelica has been used as a flavouring in gin because of its resemblance to the flavour of juniper berries. The candied leaves and stalks are used as decoration on cakes and pastries. When cooked with rhubarb. angelica reduces the tartness of the other plant. According to one source, angelica is responsible for the muscatel flavour of Rhine wines. Teas made from the roots and leaves of *A. archangelica* have been used as an expectorant, diuretic, diaphoretic, antiflatulent, and externally, to treat rheumatic and skin disorders. Angelica has been used as a remedy for respiratory ailments and in the Faeroe Islands the plant is used as a vegetable.

Chemistry

The roots and fruit of *A. archangelica* yield about 1% angelica oil, used as a flavouring and scent. Ether-pentane extracts contain volatile components including at least 16 monoterpene hydrocarbons, 13 sesquiterpene hydrocarbons, 12 monoterpene alcohols, 4 oxygenated sesquiterpenes, 11 esters, 3 lactones, 7 aliphatic carbonyls and 4 aromatic compounds. Alcohol extracts contain an additional 20 compounds.

Experimental confirmation has shown that coumarin osthol and xanthotoxin, extracts from *A. pubescens,* have significant anti-inflammatory and analgesic properties. The essential oil is known to contain alpha-phelandrene, alpha-pinene, osthenole, alpha-thujene and camphene. Root oil is considered to be of superior quality to oils obtained from other parts of the plant.

Pharmacology

Angelica contains alpha-angelica lactone, which has been shown to augment calcium binding in canine cardiac microsomes in the presence of ATP. With or without ATP, the compound also augments calcium turnover. Its action may involve increasing the contraction-dependent calcium pool to be released upon systolic depolarisation.

An attempt to find non-viral inducers of interferon failed to find any active extracts of angelica. Experimental confirmation has shown that osthole, a component of *A. pubescens* has a non-specific relaxing effect on the trachealis of guinea pigs. Volatile oil from angelica has been shown to inhibit phasic contraction of ileal muscle fibres. In contrast to some other plant extracts, angelica oil has a greater effect on tracheal tissue than on ileal tissue.

A mitogen consisting of 90% sugar and 10% protein has been found in *A. actiloba* (Kitagawa); the activity of this compound is reduced by at least half in the presence of acid or base. An aqueous extract of *A. koreana* (radix) has shown wormicidal activity against *Clonarchis*

sinensis. Alpha-angelica lactone inhibits the formation of metabolites of the carcinogen benzo (alpha) pyrene in the mouse forestomach and liver, but not in lung tissue. Volatile emissions of *A. archangelica* have demonstrated fungistatic activity against species of Aspergillus, Rhizopus, Mucor and Alternaria.

A. angelica L. has shown some antimutagenic properties in murine bone marrow cells. Anti-tumour effects on mice with Ehrlich Ascites tumours have been demonstrated by *A. sinensis* given to healthy mice promoted clone stimulating factors (CSF) in spleen conditioned medium (SCM). Sodium ferolate, an active component of *A. sinensis* Diels, exhibited hepato-protective action in mice. Ferulic acid, a phenolic compound in *A. sinensis* Diels, has inhibited uterine contractions in rats.

Toxicology
Angelica is generally recognised as safe for consumption as a natural seasoning and flavouring. The coumarins and furocoumarins may induce photosensitivity if applied topically. These compounds may also be photocarcinogenic and may be mutagenic in laboratory animals. It is possible to confuse this plant with water hemlock (*Cicuta maculata* L.) which is extremely toxic.

Summary
The term angelica applies to a number of species of herbs of the genus *Angelica*. These plants have been used for flavourings and scents, as vegetables and herbs, and in folk remedies for respiratory illnesses and arthritis. Pharmacologically, angelica contains compounds with cardiac, smooth muscle and metabolic effects, and volatile components appear to control the growth of some fungi. Topical administration of the extract may induce photosensitivity in sensitive persons.

21. BHP 1983 (B26)
ANGELICA LEAF

Definition: Angelica leaf consists of the dried leaves of *Angelica archangelica* L. (Family: Umbelliferae). Angelica leaf yields about 0.1% of volatile oil containing furanocoumarins.

Therapeutics:
Action: Carminative
Topically: anti-inflammatory
Indications: Flatulent dyspepsia. Pleurisy.
Specific Indications: Pleurisy and bronchitis applied as a compress.
Combinations used: May be combined with Chamaemelum for digestive weakness.
Preparations and dosage (thrice daily)
Dried leaf: Dose 2-5 g or by infusion. Liquid extract: 1:1 in 25% alcohol. Dose 2-5 ml. Tincture 1:5 in 45% alcohol. Dose 2-5 ml.

22. Cytostatic Activity of Coumarins in vitro

A. Gawron *1 *3, and K. Glowniak *2

Received: March 9, 1987

Abstract: The effects of coumarin fractions obtained from angelica fruits (Anchangelica

officinalis Hoffm.) und parship fruits[Pastinaca sativa L.(Apiaceae)] on the growth of cancer cells HeLa-S3 cultured in the dark were studied. It was concluded that coumarin fractions in the concentration above 5g/ml inhibited the growth of culture. The angelica coumarin fraction had a stronger effect on the growth cells than the parsnip coumarin fraction. Ten various coumarins, from which eight were isolated from fractions, showed the effects on the growth and colony forming ability of HeLa cells. The proliferation inhibition of HeLa cells by these coumarins is as follows: osthol; zanthotoxol; 4-methylaesculetin; isopimpinellin; bergapten; xanthotoxin; imperation; coumarin; umbelliferone; 4-hydroxycoumarin.

Introduction

Studies on the effect of coumarin compounds on animal organisms showed that these compounds, besides reactions such as anticoagulation, spasmolysis, and photoactivity, showed also anti-cancer properties. The ability of 8-methoxypsoralen to decrease the amount of skin cancers induced by ultraviolet radiation on white mice was described by O'Neal and Griffin (12). Some coumarins also inhibit the chemical induction of cancers (10,18). Furocoumarins, besides their ability to slow down the action of mutagenesis and carcinogenesis, also limit the increase of cancer, as has been demonstrated by laboratory reserch (17). Research on the application of coumarin in the treatment of malignant melanoma in man has also been published (16). The anti-cancer action of particular coumarin compounds can vary and it depends both on the type of compound and the type of cancer (10, 17). The angelica and the parsnip are rich source of coumarins. The contents of coumarins in angelica fruits amount to 3.5% of dry weight and include; angelicin, bergapten, 8-hydroxybergapten, imperatorin, isoimperatorin, isopimpinellin, osthenol, osthol, ostruthol, oxypeucedanin, oxypeucedanin hydrate, phellopterin, umbelliferone, umbelliprenin, xanthotoxin, and xanthotoxol (5,10). Parship fruit contain fewer cournarins (1.7% of dry weight), the coumarin fraction also contains a smaller amount of its compound types. It contains the following compounds: bergapten, coumarin, imperation, isobergapten, isopimpinellin, osthol, pimpinellin, sphondin, umbelliferone, xanthotoxin, and xanthotoxol (3,4,10). These fideings have led us to test the influence of coumarins present in these fruits on the growth of cancer cells, as well as the influence of individual compounds belonging to the simple coumarins and furocoumarin.

Materials and Methods

Coumarin fractions obtained from petroleum extracts from angelica fruits (Archangelica officinalis Hoffm.) and parsnip fruits (Pastinaca sativa L.) have been tested. Both plants were cultivated at the Medicinal Plant Garden, in the Medical Academy of Lublin. The specimens of plant material were deposited in the Department of Pharmacognosy collections. Coumarin fractions were obtained by the extraction of medium-powered fruit with petroleum. Extractions were carried out in the Quickfit extractor containing closed circulation of solvent. Semicrystalline coumarin compounds were precipitated from condensed and cooled extracts. Then coumarin fractions were separated by chromatography in order to isolate and identify compounds. Details pertaining to the isolation and identification of coumarins were described in previously published papers (5,6). Besides the bergrapten, imperatorin isopimpinellin, xanthotoxol, xanthotoxin, coumarin, umbelliferone, osthol which these fractions 'contain, 4-methylaesculatin and 4-hydroxycoumarin, which do not appear in the tested fractions, have been tested.

Tests were carried out on cultured HeLa-S3 cells. The HeLa-S3 cells were grown in Eagle's minimum essential medium (MEM 1959) supplemented with 5% inactivated calf serum and

20m/ml gentamicin. Two ml of medium containing about 10 cells were placed into Leighton's glassware. The culture was incubated at 37 degree C for 24h. After this period, the mediums was changed into a new one containing coumarin fractions at the concentrations 5, 25, and 50l/ml. The compounds were dissolved in dimethyl sulfoxide (Merck). The final concentration of DMSO in the medium was not larger than 0.5%. The medium in the control cultures contained 0.5% DMSO. After 24 and 48 h, respectively , of incubation, ten control cultures and experimental cultures were taken.

In order to compare the action of particular coumarin compounds, HeLa cells cultures were incubated with single coumarin compounds in the concentration 25g/ml for 48h. The contents of the cell protein were estimated in the samples taken according the Oyama's and Eagle's method(13). The cultures were washed three times with PBS and then dissolve in the solution of NaOH, Na2CO3, CuSO4 and sodium tartrate. Optical colour intensity of the solution containing Folin-Ciocalteau reagent was read at 650nm. For cytological studies, the cell cultures were grown on coverslips, fixed in methanol and stained with Harris's hematoxylin and eosin. The mitotic index was calculated by couting the number of mitoses and calculating per 1000 cells. The ability to form colonies was evaluated by placing about 500 cells into Petri dishes. After 24 h the medium was changed. Experimental cultures contained coumarins at the concentration of 25g/ml of the medium. The cultures were incubated for 7 days.

The atmosphere in the incubating chambers contained 5% CO2. After the incubation was completed, the colonies were fixed in methanol, counterstained with hematoxylin, and counted under a microscope. The number of colonies as well as the amount of their cells were established (single cells were also defined as colonies).

The results obtained were checked statiscally by the Student t-test, taking as the criterion of significance p &lt 0.05.

Results

The influence of angelica and parsnip coumarins on the growth of HeLa cells was evaluated by determining the contents of cell protein in the cultures. The results obtained are shown in Table I.

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24. Effects of an Active Substance Isolated from the Roots of
Angelica shkiokiana on Leukotriene and Monohydroxyeicosatetreaenoic Acid Biosyntheses in Human Polymorphonuclear Leukocytes

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Abstract: It was found that the EtOAc fraction of the roots
of Angelica shkiokiana Marino inhibited calcium ionophore-induced LTB4 and LTC4 formations in human polymorphonuclear leukocytes(PMN-L). And the active substance isolated from this root of was elucidated to be 3'(R),4'(R) -3'-epoxyangeloyloxy-4'-acetoxy-3', 4'-dihydroseselin(YN-1).
Furthermore , YN-1 at concentrations of 4-400g/ml inhibited calcium ionophor-induced 12-HETE, 5-HETE, LTB4, and LTC4 formation in human PMN-L, dose-dependently, as shown by measurements with reversed phase HPLC and radioimmunoassay systems.

Introduction

Angelica shkiokiana Marino (Umbelliferae) are cultivated in the Oita Prefecture of Japan, and are used as a substitutional drug for ginseng roots and called "Yama-Ninjin". Hata et al. (1) reported that the two coumarins anomalin (3', 4'- diangeloyloxy-3', 4'-dihydroseselin) and isopteryxin (3'- angeloyloxy-4'-acetoxy-3', 4'-dihydroseselin) were isolated from the roots of A.shkiokiana. Leukotrience are involved in immunoregulation and in a variety of diseases, including asthma, inflammation, and various allergic conditions. It was reported that leukotriene B4 (LTB4)caused lysosomal enzyme release from PMN-L in the presence of cytochalasin B (2) and had a strong chemotaxis (3). The slow-reacting substance of anaphylaxis (SRS-A) is produced in immediate hypersensitivity reactions (4) and has been shown to be composed of a mixture of LTC4, LTD4, and a small amount of LTE 4(5).

In the present work , we studied the inhibitory effects of the roots of A.shkiokiana on calcium ionophore-induced LTB4 and LTC4 biosyntheses in human polymorphonuclear leukocytes(PMN-L), and elucidated the chemical structure of the active substance from the roots.

Materials and Methods

Materials

Calcium ionophore A 23187 was purchased from Boehringer Mannheim GmbH. [H]-RIA-leukotriene B4 and C4 kits were purchased from New England Nuclear. 5-HETE methyl ester and LTB4 were obtained from Paesel GmbH Co. A reference sample of 5-HETE was obtained by hydrolysis of the methyl ester of 5-HETE. 12-HETE was isolated from human platelet metabolites of arachidonic acid. 15-HETE was also isolated from arachidinate metabolites by soybean lipoxygenase(Oriental Yeast Co.).

Chemical analysis

HPLC analysis were performed using a LKB 2152 HPLC controller. IR, UV, mass, ORD, and CD spectra were measured on a Shimadzu IR-400 spectrometer, Hitachi 220A spectrometer, JEOL JMS-HX 100 spectromer, JASCO ORD/UV-5 spectrometer, and JASCO J-500 spectropolarimeter, respectively.

H-NMR(300MHz) spectra were recorded in CDCl3 and D2O on a Varian XL-300 spectrometer. Tetramethylsilane (TMS) was used as internal standard and chemical shifts are reported on the scale (ppm and Hz). Colum chromatography was carried out using silica gel 60 (70-230 mesh, ASTM, Merck Co.) as the adsorbent.

Isolation of human polymorphonuclear leukocytes (PMN-L) PMN-L, isolated from venous blood of healthy subjects by sedimentation through 6% dexytran and Ficoll/hypade, were suspended in Hepes/saline buffer (25 mM Hepes in 135mM NACl, ph 7,4) containing 2mMCaCl2.

These cells, more than 97% of which were shown to be PMN-L by Giemsa staining and light microscopic examination, were more than 95% viable as judged by the trypan blue exclusion test.

Measurement of leukotriene B4 (LTB4) biosynthesis induced calcium ionophore in human PMN-L(RIA methods)

Human PMN-L (2.0 x 10 cells) were preincubated with the indicated amounts of test compounds for 5 min at 37 degree C. Then, 2M of calcium ionophore were added and the mixture was incubated for 5 min at 37 degree C in a final volume of 0,4 ml. The reaction was stopped by adding 0.6 ml of ice-cold 10 mM Tris-HCl buffer (pH8.6) containing 0.9% NaCl, 0.1% gelatin, and 0.1% sodium azide. The incubation mixture was centrifuged at 1000 x g and 4 degree C for 15 min,and LTB4 in the supernatant was determined with a leukotriene B4 [3H]-radioimmunoassay (RIA) kit. Measurement of leukotriene C4 (LTC4) biosynthesis induced by calcium ionophore in human PMN-L LTC4 induced by calcium ionophore was assayed by the method of Conroy et al. (6). Briefly, human PMN-L (2.8 x 10 cells) were preincubated with the indicated amounts of test compounds and 10 mM cysteine for 5 min at 37 degree C. Then, 2M of calcium ionophore were added and the mixture was incubated for 10 min at 37 degree C in a final volume of 0.4 ml. The reaction was stopped by adding 0.6 ml of ice -cold Hepes (saline buffer(pH7.4). The incubation mixture was centrifuged at 1000 x g and 4 degree C for 15 min and the supernatant was applied on a Sep-Pak C18 cartridge(Waters Co.) The Sep-Pak column was washed with acetate buffer (pH 5.6) and H2O, and eluted with MeOH. The elude with MeOH was evaporated under N2 at 40 degree C , and the residue was dissolved in a small amount of MeOH(20l). Reversed phase high performance liquid chromatograohy (HPLC) of the partially purified LTC4 fraction was performed on a column (4.6 x 250 mm) of Nucleosil C18 (5m, particles, Macherey-Nagel Co., Duran, Germany) using ultraviolet (UV) absorption measurement at 280 nm for detoction. The reversed phase HPLC analysis was carried out by developing with a mixture of MeOH-H2O-AcOH (60:40:0.06,v/v) at a flow rate of 1.0ml/min under a pressure of 120-123 bar.(Fig.1). LTC4 fractions collected by reversed phase HPLC were evaporated under N2 and LTC4 contents were determined with a leukotrience C4 [H] -RIA kit.

Fig.1. Reversed phase HPLC chromatogram of a reference sample of LTC4. Separation achieved on a Nucleosil C18 (4.6 x 250mm) eluted with MeOH -H2O -AcOH (60:40:0.06, v/v) at a flow rate of 1.0 ml/min and 280 nm for detection.

Measurement of isomic LTB4, LTB4,15-HETE, 12-HETE, and 5-HETE biosyntheses induced by calcium ionophore in human PMN-L (Reversed phase HPLC methods)

Human PMN-L (2.0 x 10cells) were preincubated with the indicated amounts of test compounds for 5 min at 37 degree C. Then, 2M calcium ionophore was added and the mixture was incubated for 5 min at 37 degree C in a final volume of 0.4 ml. The reaction was stopped by adding 0.2 ml of 0.5 N HCOOH and the mixture was extracted with 8 volumes of EtOAc. The EtOAc phase was evaporated under N2. The residue was dissolved in a small amounts of MeOH (40l). Isomeric LTB4, LTB4, 15HETE, 12-HETE, and 5-HETE formed from PMN-L

were determined by reversed phase high performance liquidid chromatography (HPLC).

This was performed on a YMC-pack ODS (A 312, Yamamura Chemical Lab.) (6 x 150 ml) using UV absorption measurement at 280 nm (retention time; 0-12 min) and 240 nm (retention time ; 12-40 min) for detection. The analysis of leukotrienes and HTETs was carried out by developing with a mixture of CH3CN-H2O-MeOH-AcOH (150:110:50:0.02, v/v) at a flow rate of 1.5 ml/min under a pressure of 65-68 bar. The activities of YN-I isolated from the roots of A. shiokiana are expressed as percentage as compared to each control value. YN-1 Colorless, viscous oil. It exhibited a blue -violet fluorescence under a UV lamp. High resolution mass spectrum m/z : 402.1335 (MC21H 22 O8)(10%), 360(2%), 245(47%), 244(52.5%), 229(base ion peak) and 43(15%), Yield 3.1g.UV (log)nm: 207(4,53), 217(sh.)(4.26), 245(3.72), 255(3.65), 301(sh.)(4.06), 324(4.24). IR cm; :1730 (broad)(C =O), 1600(aromatic ring). ORD(c=1.09,EtOH) [] (nm); -27.5(589), -36.7(550), -45.9(500), -49.5(445), -31.2(400), 0(380), +77.1(360). CD(c=2.74 x 10 , EtOH) (nm): 0(350), +1.33(330), +1.77(325)(positive max.), +0.66(280), 0(260), -3.10(250), -3.32(245)(negative max.), -3.10(240), 0(230), +8.18(223)(positive max.), +5.75(220), 0(216). H-NMR (CDCl3) ppm; 1.39[3H, d, J=5.6 Hz, -CH(-O-)-CH3], 1.44, 1.48[3H x 2,s, (CH3) 2C &lt], 1.57[3H,s, -C(-O-)-CH3], 2.14(3H, s, -OCO-CH3), 3.07 [1H, q, J=5.6 Hz, &gt CH(-O-)-CH3], 5.40 6.59 (1H x 2, d, J=5.0 Hz, O-CH-CH-O), 6.25, 7.61 (1H x 2,d, J=9.4 Hz, -CH=CH-), 6.82, 7.37 (1H x 2, d, J=8.5 Hz, aromatic H x 2). Saponification of YN-I with NaOH (EtOH): Formation of YN-Ia, YN-Ib, YN-Ic, and YN-Id. To the solution of 200 mg of YN-I in 30 ml of EtOH, 10ml of 1 N NaOH(EtOH) was added and the mixture was allowed to stand at room temperature for 15 mn. Then the reaction was stopped by addeing 200ml of H2O, acidified with 20% H2SO4, and extracted with Et2O. The Et2O solution was washed with 5% Na2CO3 and evaporated. The residue upon chromatograph over silica gel (20 g) with n-hexane- EtOAc(3:1) gave four products YN-Ia, YN-Ib, YN-Ic, and YN=Id. YN-Ib, YN-Ic and YN-Id were identified by comparison with authentic samples of (+)-cis-ethylkhellactone, (-)-trans-ethylkhellactone, and (+) -cis-khellactone by comparison of the spectral data. YN-Ia, colorless, viscous oil. H-NMR (CDCl3) ppm: 1.28[3H,d,J=5.5 Hz, -CH(-O-)-CH3], 1.30(3H,t,J=7.0Hz, O-CH2-CH3),1.47,1.15, [H x 2, s, &gt C(CH33)2], 1.52[3H,s,H3C-C(-O-) &lt], 2.99[1H,q, J=5.5Hz, -CH(-O-)-CH3], 4.02(2H, m, O-CH2-CH3-), 4.47, 5.27(1H, x 2, d, J=2.1 Hz, O-CH-CH-O), 6.26, 7.63(1H x 2, d, J=9.6 Hz, -CH = CH-), 6.81, 7.34(1H x 2,d, J= 8.5 Hz, aromatic H x 2).

Cytotoxicity

Various extracts and YN-I used in this experiment did not cause PMN-L suspensions to release more than 8% of their lactate dehydrogenase and, therefore, were not toxic to the cells (data not shown).

Results

Isolation of the active substance

The dried and crushed roots (1kg) of the plant collected in the Oita Prefecture of Japan were successively extracted with EtOAc and MeOH (3 x 21) for 3h under reflux, respectively. The residue was further extracted with H2O(51) at room temperature for 1 week. The EcOAc, MeOH, and H2O extracts were concentrated in vacuo to give 9.5g, 51.3g, and 54.5g resideues, respectively. The EtOAc extract (9.0g), inhibiting LTC4 formation in human PMN-L, was chromatographed on a column of silica gel (200g) with n-hexane-EtOAc (3:2) as the eluent.

The part eluted with n-hexane-EtOAc (3:2) was divided into five fractions, Fr. 1 (558mg), Fr 2 (222mg), Fr 3 (5.60g), Fr 4 (85mg), and Fr.5(383mg). Fr 3 (5.3g) was rechromatographed on a silica gel (150 g) column with CHCl3- EtOAc (20:1) to give an achieve substance (YN-I).

Effects of the various fractions from the roots of A. shkiokiana on LTB4 and LTC4 formations induced by calcium ionophore in human PMN-L (RIA methods)

As shown in Fig. 2, the EtOAc fraction of the roots of A. shkiokiana inhibited the calcium ionophore-induced LTB4 and LTC4 formations in human PMN-L.

Effects of the active substance (YN-I) isolated from the roots of A. shkiokiana on calcium ionophore-induced LTB4 and LTC4 in human PMN- L (RIA methods)

As shown in Fig. 3, YN-I inhibited LTB4 and LTC4 formations dose-dependently in human PMN-L at concentrations of 4-400g/ml. The 50% inhibitory concentrations (IC50) for LTB4 and LTC4 were 7.2g/ml and 4.8g/ml, respectively.

Fig.2a.and b. Effects of EtOAc, MeOH, and H2O extracts on calcium ionophre-induced LTB4 and LTC4 formations in human PMN-L. Values are means for 2 experiments, , EtOAc ext; C, MeOH ext; , H2 ext. Fig.2a. Fig.2b. Fig.3a. Fig.3b. Fig.3a.and b.

Effects of YN-I on calcium ionophore-induced LTB4 and LTC4 formations in human PMN-L.

Values are means S.E, for 3 experiments.

Effects of YN-I on 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE), 15-hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE), 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE), and LTB4 biosyntheses induced by calcium ionophore in human PMN-L (reversed phase HPLC methods)

As shown in Fig. 4a, the peaks 1 and 2, peak 3, peak 4, and peak 5 were identified as two LTB4 isomers, LTB4, 15-HETE, 12-HETE and 5-HETE by their retention time on reversed phase HPLC and by coelution with known standards. Fig. 4a shows that YN-I inhibited the formations of 12-HETE, 5-HETE, LTB4 isomers, and LTB4 at concentrations of 4 g/ml and 40g/ml. As shown in Fig. 4b, YN-I at concentrations of 0.4-40g/ml inhibited the formations of 12-HETE, 5-HETE, and LTN4, dose-dependently. But 15-HETE formation was not affected. The IC50 values for LTB4, 12-HETE, and 5-HETE formations were 7.8 g/ml, and 12.0g/ml, respectively.

Fig.4a.

Fig.4a. Reversed phase HPLC chromatogram of metabolites induced by calcium ionophore in human PMN-L in the presence of abesence of YN-I. (A) Calcium ionophore alone (control); (B) Calcium ionophore plus YN-I (40g/ml) (C) Calcium ionophore plus YN-I 40g/ml). Peaks 1 and 2, LTB4 isomer; peak 3, LTB4; peak 4, 15-HETE; peak 5, 12-HETE; peak 6, 5-HETE. Separation achieved on a YMO pack ODS column (6 x 150 mm) eluted with CH3CN-MeOH-H2 -AcOH(150:50:0.02,v/v) at a flow rare of 1.5 ml/min.

Fig.4b.

Fig.4b. Effects of YN-I on LTB4, 15-HETE, 12-HETE, and 5-HETE formations induced by calcium ionophore in human PMN-L. Values are means S.E. for 3 experiements. , LTB4; , 5-HETE; , 12-HETE; , 15-HETE.

Discussion

Effects of the various fractions and an active substance (YN-I) isolated from the roots of A. shkiokiana on leukotriene and HETE biosyntheses induced by calcium ionophore in human PMN-L In the present work, we studied the effects of three fractions (EtOAc, MeOH, and H2O fractions) and YN-I, isolated from the roots of A. shkiokiana, on leukotrienes (two LTB4 isomers, LTB4 and LTC4 and HETEs 15-HETE, 12-HETE and 5-HETE) induced by calcium ionophore in human PMN-L by the methods of reversed phase HPLC or RIA. As shown in Fig. 4a, two LTB4 isomers (peak 1 and 2), LTB4 (peak 3), 15-HETE (peak 4), 12-HETE (peak 5), and 5-HETE (peak 6) were detected by reversed phase HPLC. However, each peak (corresponding to two LTB4 isomers, LTB4, 12-HETE, and 5-HETE) was founded to cross over to that of the extracts of A. shkiokiana (data not shown). Furthermore, the peak corresponding to LTC4 by reserved phase HPLC also crossed over to that of the extracts of this drug at 30-35 min of retention time. YN-I isolated from this drug also crossed over to the peak of LTB4 formed from PMN-L at a high concentration (400g/ml). Therefore, the measurements of LTB4 and LTC4 formed from PMN-L were estimated by the methods of RIA. And the measurements of the actions of YN-I on LTB4, 15-HETE, 12-HETE, and 5-HETE formations in human PMN-L were carried out by reversed phase HPLC analysis at concentrations of 0.2-40g/ml of YN-I. (Fig. 4a) Calcium ionophore is through to induce SRS biosynthesis in leukocytes by activating the Ca-dependent hydrolysis of membrane phospholipids, liberating arachidonic acid, which is further metabolized by 5-lipoxygenase, initiating the pathway to SRS formation(4). In the present work, we found that an active substance, YN-I, was isolated from the roots of A.shkiokiana, and that YN-I inhibited the formations of 12-HETE, 5-HETE, LTB4, and LTC4 induced by calcium ionophore. But 15-HETE formation was not affected. YN-I could inhibit LTB4, LTC4, and 5-HETE formations by one or more of three mechanisms; firsrly, inhibition at the level of calcium ionophore-induced Ca influx through cell membranes; secondly, inhibition of a 5-lipoxygenase step subbsequent to Ca influx; and thirdly, inhibitation of phospholipase A2 , thus preventing the release of arachidonic acid from membrane phospholipids. The three possible mechanisms for the inhibitions of LTB 4,LTC4, and 5-HETE formations require further work for clarificantion.

The chemical structure of an active substance (YN-I) YN-I, a colorless, viscous oil, C21H22O8 (M 402.1335), exhibited a blue-violet fluorescence under UV lamp. The infrared (IR) spectrum of YN-I showed the presence of carbonyl and aromatic ring moities. The ultraviolet (UV) spectrum of YN-I exhibited similarities to that of 7-hydroxycoumarin derivatives (7,8). The optical rotary dispersion (ORD) spectrum of YN-I showed a positive plain curve. The proton nuclear magnetic resouance (H-NMR), spectrum (CDCL3) shows two pairs of doublet with intensities corresponding to one proton each, one pair appearing at = 6.25 and 7.61 ppm (J= 9.4Hz) can be assigned to protons at the 3- and 4-positions of the coumarin ring and the other at = 6.82 and 7.37 ppm (J= 8.5Hz) for the 5- and 6-positions of the coumarin ring, respectively. Further signals are obsweved at = 5.40 and 6.59 ppm (each 1H, d, J=5.0Hz), at 3.07(1H,q, J=5.6 Hz) and 1.39 (3H, d, J=5.6 Hz) as well as signals due to an acetoxyl group at 2.14 (3H, s), gem-dimethyl groups at 1.44 and 1.48 (each 3H,s), and a methyl group at 1.57 ppm (3H,s,). As shown in Fig.3, the mass fragments of YN-I showed 402 (M), 360 (M-O=C=CH 2), 245 (M -2,3 -dimethyloxiran-2-oyloxy), 244 (M+ -2,3-dimethyloxiranc-2-carboxylic acid), 229 (base ion peak), and 43 (COCH3), respectively. These data of YN-I are very similar to those of

epoxypteryxin[3' (R), 4' (R), 3'-acetoxy-4'epoxyangeloyloxy3',4'-dihydroseselin] isolated from the roots of Laserpitium archangelica Wulf. (9). Therefore, the structure of YN-I seems to be either 3'-epoxyangeloyloxy-4'-acetoxy-3',4'-dihydroseselin or 3'-acetoxy-4'epoxyangeloyloxy-3',4'-dihydroseselin.

Saponification of YN-I with 1 N-ethanolic sodium hydroxide in room temperature afforded four products: YN-Ia, YN-Ib, YN-Ic, and YN-Id. Amount them, YN-Ib, YN-Ic, and YN-Id were identical with (+)-cis-ethylkhellactone, (-)-trans-ethylkjellactone, and (+)-cis-khellactone by comparison of the spectral data with those of authentic samples,respectively. The HNMR spectrum of YN-Ia was very similar to that of trans-ethylkhellanctone except for the fact that epoxyangeloyl group signals at = 2.99(1H, q, J=5.5 Hz), 1.52(3H, s), and 1,28ppm(3H, d, J=5.5 Hz) and an oxymethine signal shifted to down field at = 5.27ppm(1H, d, J=2.1 Hz) were observed instesd of an oxymethine signal at = 3.90 ppm(1H, br.s) and a hydroxy group signal at = 2.60ppm(1H, br.s). Consequently, YN-Ia was deduced to be trans-3'-epoxyangeloyloxy-4'ethoxy-3', 4'-dihydroseselin.

Therefore, YN-I was concluded to be 3'-epoxyangeloyloxy-4'-acetoxy-3', 4'-dihydroseselin. The relative configuration of the C-3' and C-4' positions of YN-I were determined to be cis by observation of the coupling constant between 3'-H and 4'-H(J=5.0Hz) in the H-NMR spectrum. Moreover, the absolute configuration of YN-I was concluded to be 3' (R) and 4' (R) from the the evidence that (+)-cis-ethylkhellactone and (-)-trans-ethylkhellactone were obtained by saponification of YN-I and that the ORD spectrum of YN-I showed a positive plain curve and that the circular dichroism (CD) spectrum of YN-I showed multi-Cotton effects very similar to that of anomalin[3'(R), 4'(R)-3',4'-diangeloyloxy-3', 4'-dihydroseselin] (10,11). Accordingly YN-I was established to be 3' (R), 4' (R)-3'-epoxyangeloyloxy-4'-acetoxy-3', 4'-dihydroseselin.

Fig.5.

Fig.6.

Conclusion

Recently, the roots of Angelica shkiokiana have been used in treatment of adult diseases such as hyperlipemia and diabetes, inflammatory and allergic diseases as a substitutional drug for ginseng roots in the Kyushu region of Japan. In the present work, we isolated a coumarin devivative 3' (R), 4'(R)-3'epoxyangeloyloxy-4'-acetoxy-3',4'-dihydroseselin from the roots of A. shkiokiana as an anti-allergic substance. The present results suggest that 3' (R), 4' (R)-3'epoxyangeloyloxy-4'-acetoxy-3', 4'-dihydroseselin isolated from these roots may be effective as drug for use in treatment of allergic diseases such as atopic dermatitis and asthma. The clinical effectiveness requires evaluation.

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