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Molecules of Interest

Usnic acid

K. Ingólfsdóttir*

Faculty of Pharmacy, University of Iceland, Hagi, Hofsvallagata, 107, Reykjavik, Iceland

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Abstract

Since its first isolation in 1844, usnic acid [2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzo-furandione] has become the most extensively studied lichen metabolite and one of the few that is commercially available. Usnic acid is uniquely found in lichens, and is especially abundant in genera such as *Alectoria*, *Cladonia*, *Usnea*, *Lecanora, Ramalina* and *Evernia*. Many lichens and extracts containing usnic acid have been utilized for medicinal, perfumery, cosmetic as well as ecological applications. Usnic acid as a pure substance has been formulated in creams, toothpaste, mouthwash, deodorants and sunscreen products, in some cases as an active principle, in others as a preservative. In addition to antimicrobial activity against human and plant pathogens, usnic acid has been shown to exhibit antiviral, antiprotozoal, antiproliferative, anti-inflammatory and analgesic activity. Ecological effects, such as antigrowth, antiherbivore and anti-insect properties, have also been demonstrated. A difference in biological activity has in some cases been observed between the two enantiomeric forms of usnic acid. Recently health food supplements containing usnic acid have been promoted for use in weight reduction, with little scientific support. The emphasis of the current review is on the chemistry and biological activity of usnic acid and its derivatives in addition to rational and ecologically acceptable methods for provision of this natural compound on a large scale.

Keywords: Usnic acid; Lichen; Biological activity; Antimicrobial; Antiprotozoal; Antiviral; Antiproliferative; Anti-inflammatory; Dermatitis

1. Introduction

Of the hundreds of known secondary lichen metabolites, the dibenzofuran derivative usnic acid (**1**) is without a doubt the most extensively studied. Lichens are formed through symbiosis between fungi (mycobionts) and algae and/or cyanobacteria (photobionts). The morphology of these organisms can differ perceptibly, from crustose lichens growing on rocks to foliose or fruticose lichens growing on tree trunks, soil or other substrates. A number of the 17,000 known species have been utilized by mankind, e.g. for dyeing, pollution monitoring, perfumery, floral decorations, as well as for dietary and medicinal purposes.

Chemotaxonomic studies have shown that the most unique lichen metabolites belong to the chemical classes of depsides, depsidones and dibenzofurans. Slow growth, and often harsh living conditions, make production of protective metabolites a necessity to lichens, and many secondary constituents are believed to serve as antigrowth, antimicrobial or antiherbivore agents.

2. Chemistry and biosynthesis of usnic acid

Details of the isolation of usnic acid (**1**), along with observations on basic physical and chemical characteristics, were first described during the formative years of organic- and phytochemistry (Knop, 1844). Usnic acid [2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzo-furandione; $C_{18}H_{16}O_7$], is a yellow cortical pigment and occurs in two enantiomeric forms, depending on the projection of the angular methyl group at the chiral 9b position (Fig. 1). The absolute configuration of (+)-usnic acid at 9b has been determined by X-ray analysis to be R (Huneck et al., 1981). Of plausible tautomers, i.e. two 1,3-keto-enolic- and a 1,3-dioxo-form, the low energy 1-oxo, 3-hydroxy confirmation (**1**) is preferred according to AM1 calculations (Correché et al., 1998). Of the three hydroxyls present in the molecule, the enolic 3-OH has the strongest acidic character (pK_a 4.4) due to an inductive effect of the keto group.

* Tel.: +354-525-4000; fax: +354-525-4071.
E-mail address: kring@hi.is (K. Ingólfsdóttir).

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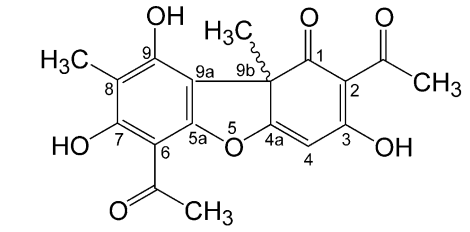

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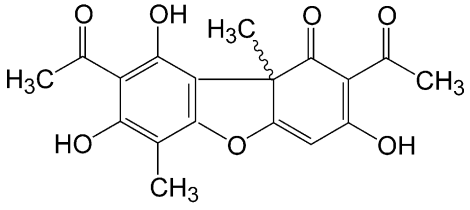

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Fig. 1. Structures of (+)-(9b-R)- and (−)-(9b-S)-usnic acids (**1**) and (+)-(9b-R)- and (−)-(9b-S)-isousnic acids (**2**).

The acidity of the phenolic 9-OH (pK_a 8.8) is enchanced by an inductive effect of the *para*-located acetyl group in postion 6, while the phenolic 7-OH is weakly acidic (pK_a 10.7), possibly due to its engagement in intramolecular hydrogen bonding to the 6-acetyl group (Sharma and Jannke, 1966).

In addition to (+)- and (−)-usnic acids, two other natural isomers, (+)- and (−)-isousnic acids (**2**), also occur in lichens. The latter are structural isomers of the parent compound, differing in substitution pattern in ring A (Fig. 1).

The biosynthesis of usnic acid (Fig. 2) proceeds via acetate to polyketide to methylphloracetophenone, with incorporation of the C_1 fragment occurring prior to aromatisation (Taguchi et al., 1969). Subsequent steps involve stereospecific oxidative phenolic coupling of two methylphloracetophenone units to give hydrated usnic acid and finally dehydration leading to ether linkage formation.

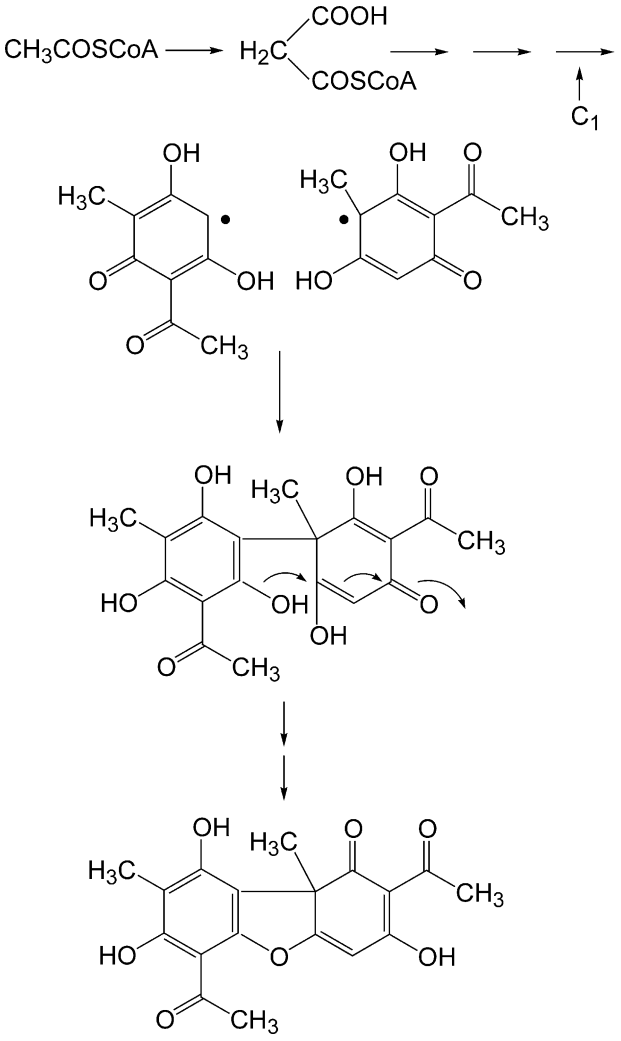

Fig. 2. Proposed biosynthetic route for usnic acid (Taguchi et al., 1969).

3. Distribution of usnic acid

Usnic acid is widely distributed in species of *Cladonia* (Cladoniaceae), *Usnea* (Usneaceae), *Lecanora* (Lecanoraceae), *Ramalina* (Ramalinaceae), *Evernia*, *Parmelia* (Parmeliaceae) and other lichen genera. *Alectoria* (Alectoriaceae) species are often rich sources of usnic acid, and yields of up to 6% have been reported (Proksa et al., 1996). In the literature usnic acid has been quoted as being present in *Cetraria islandica* (Parmeliaceae), commonly known as Iceland moss or *Lichen islandicus*. In our extensive studies on *C. islandica* from different locations within Iceland, however, we have never come across usnic acid, even in trace amounts.

Although usnic acid has only been identified in lichens, closely related compounds have been found in fungi, e.g. the phytotoxin mycousnine and similar compounds in *Mycosphaerella nawae* (Sassa and Igarashi, 1990) and cercosporamide and usnic acid amide in *Cercosporidium henningsii* (Conover et al., 1992).

4. Medicinal use of lichens containing usnic acid

Lichens belonging to usnic acid-containing genera have been used as crude drugs throughout the world. Many *Cladonia* species were used in the treatment of pulmonary tuberculosis (Vartia, 1973) and *Usnea* species have been used in Asia, Africa and Europe for pain relief and fever control (Okuyama et al., 1995). *U. barbata* was allegedly used by Hippokrates to treat urinary complaints and *U. longissima* ("Sun-Lo") by the Chinese in wound healing and as an expectorant (Shibata et al., 1948). Extracts of *U. barbata* have been used

as a source of usnic acid in modern-day cosmetic and pharmaceutical preparations. In Argentina *U. densirostra*, known as "Barba del la Piedra", is sold for various disorders (Correché et al., 1998) and *Ramalina thrausta* was used in Finland externally for treating wounds, athlete's foot or other skin complaints and orally to treat sore throat and toothache (Vartia, 1973).

Although many of the reputed effects mentioned above have neither been substantiated nor formally attributed to usnic acid, the indications have served as a guide for pharmacological studies of these lichens and usnic acid.

5. Biological activity of usnic acid

5.1. Antimicrobial and antiprotozoal activity

Indications of usnic acid being a potentially interesting candidate for antimicrobial testing (Table 1) followed reports of both optical antipodes being active against Gram positive bacteria and mycobacteria (Shibata et al., 1948; Stoll et al., 1950). These findings followed the discovery of the penicillins, as the search for potentially effective antimicrobial agents was extended to organisms other than fungi. Reviews of early antimicrobial screening of lichen extracts and isolated compounds can be found in the literature (Vartia, 1973).

The current threat of multidrug-resistant tubercular strains prompted the testing of (+)-usnic acid for activity against *Mycobacterium aurum*, a non-pathogenic organism with a similar sensitivity profile to *M. tuberculosis*, using modern standardized methods (Ingólfsdóttir et al., 1998). Although activity was confirmed, the MIC value of 32 μg/ml was not considered potent enough to merit further studies.

The in vitro susceptibility of pathogenic Gram positive and anaerobic bacteria towards (+)- and (−)-usnic acids has been confirmed using standardized assays

Table 1
Antimicrobial activity of (+)- and (−)-usnic acids. In cases where quantitative data are available, MIC values are expressed in μg/ml. + Positive activity defined qualitatively; – Inactive; n.d. Not determined

Organism	(+)-Usnic acid	(−)-Usnic acid	Reference
Gram positive bacteria			
Enterococcus faecalis	4	8	Lauterwein et al. (1995)[a]
Enterococcus faecium	16	16	Lauterwein et al. (1995)[a]
Staphylococcus aureus	6/6/85/ 8	3/6/120/8	Shibata et al. (1948)/Stoll et al. (1950)/Ghione et al. (1988)/Lauterwein et al. (1995)[a]
Streptococcus mutans	15	85	Ghione et al. (1988)
Streptococcus pneumonia	−	−	Lauterwein et al. (1995)[b]
Streptococcus pyogenes	4/35	4/100	Stoll et al. (1950)/Ghione et al. (1988)
Gram negative bacteria			
Escherichia coli	−/−/−	−/−/−	Stoll et al. (1950)/Ghione et al. (1988)[c]/Lauterwein et al. (1995)[b]
Haemophilus influenzae	−		Lauterwein et al. (1995)[b]
Pseudomonas aeruginosa	–	–	Lauterwein et al. (1995)[b]
Anaerobic bacteria			
Bacteroides fragilis	2	1	Lauterwein et al. (1995)
Bacteroides ruminicola ssp. *brevis*	8	16	Lauterwein et al. (1995)
Bacteroides thetaiotaomicron	4	8	Lauterwein et al. (1995)
Bacterioides vulgatus	4	8	Lauterwein et al. (1995)
Clostridium perfringens	4	4	Lauterwein et al. (1995)
Propionibacterium acnes	2	2	Lauterwein et al. (1995)
Mycobacteria			
M. aurum	32	n.d.	Ingólfsdóttir et al. (1998)
M. avium	6/31	6/8	Shibata et al. (1948)/Stoll et al. (1950)
M. smegmatis	20	8	Stoll et al. (1950)
M. tuberculosis var. *bovis*	2.5–4	2.5	Stoll et al. (1950)
M. tuberculosis var. *hominis*	2.5–5	2.5–16	Stoll et al. (1950)
Yeast/Fungi			
Candida albicans	−/−	−/−	Ghione et al. (1988)[c]/Lauterwein et al. (1995)[b]
Fusarium moniliforme	+	n.d.	Cardarelli et al. (1997)[d]
Penicillium frequentans	n.d.	+	Proksa et al. (1996)[e]
Saccharomyces cerevisiae	−/−	−/−	Stoll et al. (1950)/Lauterwein et al. (1995)[b]
Verticillium albo-atrum	n.d.	+	Proksa et al. (1996)[e]

[a] Numerous clinical isolates and standard strains; MIC_{50} presented (MIC at which 50% of strains are inhibited).
[b] Inactive at concentrations $\leqslant$32 μg/ml (maximum achievable concentration using microdilution method).
[c] Inactive on basis of MIC $>>$ 100 μg/ml.
[d] 50% reduction in mycelial growth diameter at concentration of 100 μg/ml.
[e] Disc diffusion method; activity determined at concentration of 100 μg/disc

(Table 1). The two isomers have been shown to exhibit activity against clinical isolates of *Enterococcus faecalis* and *E. faecium* and clinical isolates of *Staphylococcus aureus*, including strains resistant to methicillin and mupirocin (Lauterwein et al., 1995). The isomers also showed significant activity against pathogenic anaerobic Gram negative bacilli (*Bacteroides* spp.) and anaerobic Gram positive bacteria, i.e. *Clostridium* and *Propionibacterium* species. In most cases the isomers exhibited comparable activity, but (+)-usnic acid was more active against *E. faecalis* and some of the *Bacteroides* species (Table 1).

In an earlier study (Ghione et al., 1988), (+)-usnic acid was found to exert superior activity against *Streptococcus mutans* isolated from human dental lesions as compared to (−)-usnic acid (Table 1). Due to the role of *S. mutans* in the etiology of dental caries and periodontal disease, trials were performed whereby mouthwash containing 1% (+)-usnic acid was administered to volunteers and samples of oral bacterial flora subsequently taken at regular time intervals. Results showed that *S. mutans* growth was selectively suppressed without equilibrium of other oral bacteria being substantially affected.

In a follow-up study (Grasso et al., 1989) the effects of an (+)-usnic acid containing toothpaste on *S. mutans* were assessed. Analysis of bacterial samples taken daily before and after brushing showed a significant decrease in the number of colony forming units of *S. mutans*.

Hydrazone derivatives, prepared by the condensation of usnic acid with hydrazides of α-naphthoic-, caprylic- and oxamic acids, did not show improved antibacterial activity as compared to that of the parent compound (Sladić et al., 1998). Interestingly, however, on chelation of the hydrazones with Cu(II), antibacterial activity was enhanced. Thus, the Cu(II)-complexes exhibited activity against *Escherichia coli*, whereas usnic acid and the parent ligands were inactive, and activity against *S. aureus* was in some cases enhanced compared to usnic acid (Beljanski et al., 1998).

Antifungal activity (Table 1) has been ascribed to (−)-usnic acid against the plant pathogens *Penicillium frequentans* and *Verticillium albo-atrum* (Proksa et al., 1996). A diastereomeric mixture of dihydrousnic acids showed a broader spectrum of activity, inhibiting growth of *P. cyclopium*, *P. frequentans*, *Talaromyces flavus* and *Trichosporon cutaneum* while the-1-phenyl- and-1-(*N*-isonicotinoyl)- hydrazones were inactive.

Ethoxydiglycol extracts of lichens, standardized to contain 10% wet wt. usnic acid, have been shown to have preservative potential in moisturizing cream (Seifert and Bertram, 1995). On account of effects vs. Gram positive organisms, mainly responsible for the development of body odour, usnic acid has been commercially used in deodorant sprays. To render the compound soluble and stable for such formulations a complex was produced by reacting usnic acid with triethanolamine (Bergerhauser, 1976).

Chinese researchers have shown (−)-usnic acid to exhibit inhibitory effects in vitro against the pathogenic protozoan *Trichomonas vaginalis* at slightly lower concentrations than metronidazole (Wu et al., 1995).

5.2. Antiviral activity

Commercially obtained (+)-usnic acid was shown to inhibit cytopathic effects of Herpes simplex type 1 and polio type 1 viruses when administered on filter paper discs which were placed on virus-infected African green monkey kidney (BS-C-1) cells (Perry et al., 1999).

In a clinical study recently performed in Italy with the participation of 100 female patients (aged 18–45) infected with genital human papillomavirus, the effects of an intravaginal formulation containing usnic acid and zinc sulphate were evaluated for use in adjuvant therapy to radiosurgical treatment (Scirpa et al., 1999). Results showed significantly favourable results for patients receiving the Zn–usnic acid vaginal formulation, both with regard to re-epithelization of lesions and recurrence of infection over a 6 month period, as compared to a control group receiving no adjuvant treatment. The Zn–usnic acid formulation was generally well tolerated, but local irritant effects were experienced by 8% of the patients.

In a cancer chemoprevention assay designed to detect potential inhibitors of tumour promotion, (+)-usnic acid isolated from *U. longissima* showed potent inhibitory effects (ED_{50} 1.0 μg/ml) against Epstein–Barr virus activation induced by teleocidin B-4, a potent tumour promoter (Yamamoto et al., 1995). Commercially obtained (−)-usnic acid was less active (ED_{50} 5.0 μg/ml).

5.3. Antiproliferative activity

(−)-Usnic acid has been shown to exhibit moderate activity in the murine P388 leukaemia assay and in vitro cytotoxic activity against cultured L1210 cells (Takai et al., 1979). Attempts were made to prepare derivatives with more potent activity by disrupting the strong intramolecular hydrogen bonds so as to decrease lipophilicity of the molecule (Takai et al., 1979). None of the 11 synthetic derivatives however exhibited more potent activity than the parent compound, and the β-triketone moiety was considered vital for maintaining activity.

(+)-Usnic acid (50 μg/ml) was shown to reduce cell counts of leukemic (K-562) and endometrial carcinoma (Ishikawa, HEC-50) cell lines when exposed to the cultures for 21 h. Extending the exposure time to 46 h caused inhibition to increase significantly, indicating the importance of the time factor (Cardarelli et al., 1997). In a recent investigation (+)-usnic acid solubilized in

2-hydroxypropyl-β-cyclodextrin exhibited antiproliferative activity against the malignant K-562 cell line in a standard thymidine proliferation assay with an ED_{50} of 4.7 μg/ml (Kristmundsdóttir et al., 2002).

In studies aimed at screening natural compounds for potential to combat abnormal proliferation of keratinocytes experienced in psoriasis, (+)-usnic acid showed antiproliferative activity against the human keratinocyte cell line HaCaT with an IC_{50} value of 2.1 μM (Kumar and Müller, 1999). The effects were not considered to be through membrane damage and were judged to be cytostatic rather than cytotoxic.

5.4. Anti-inflammatory activity

Recent studies in which the anti-inflammatory activity of (+)-usnic acid was compared to that of ibuprofen using the rat paw oedema assay (acute effects) and the cotton pellet assay (chronic effects) showed (+)-usnic acid to be significantly effective in both assays at an oral dose of 100 mg/kg and comparable to ibuprofen at the same dose (Vijayakumar et al., 2000). In an earlier study (+)-usnic acid had shown significant activity in the cotton pellet assay in rats at an oral dose of 50 mg/kg (Ôtsuka et al., 1972).

5.5. Analgesic and antipyretic activity

Usnic acid and the depside diffractaic acid were identified as active components of a *U. diffracta* methanol extract showing analgesic and antipyretic activity in mice (Okuyama et al., 1995). Oral administration of usnic acid at 30 and 100 mg/kg resulted in significant analgesic effects as determined by the acetic acid-induced writhing- and tail pressure tests. Usnic acid administered orally at doses of 100 and 300 mg/kg exhibited significant antipyretic activity as evaluated through lipopolysaccharide-induced hyperthermia.

6. Ecological effects

Numerous investigations have highlighted the importance of secondary lichen metabolites in chemical ecology by demonstrating lichen–microbe, lichen–plant and lichen–animal interactions. Apart from ecological significance these investigations have also been considered from a commercial viewpoint, i.e. development of agricultural antimicrobial agents, herbicides and insecticides. Coverage of the ecological effects reported for usnic acid will be restricted here to a few examples focusing on enantiospecific effects.

In addition to growth inhibitory effects of usnic acid against plant pathogens mentioned above (Section 5.1), the compound has been shown to reduce mycelial growth of *Fusarium moniliforme*, albeit at high concentration (Table 1). Antimitotic effects of usnic acid on cultured plant cells (Cardarelli et al., 1997) and capacity to inhibit germination and growth of higher plants is well known. In a recent study, in which both enantiomers were tested for phytotoxicity and compared to commercial herbicides, (−)-usnic acid proved significantly more potent. The mechanism of activity was attributed to inhibition of *p*-hydroxyphenylpyruvate dioxygenase (HPPD), (−)-usnic acid exhibiting an IC_{50} level of 50 nM (Romagni et al., 2000).

Although both usnic acid enantiomers caused significant antifeedant activity and toxicity towards larvae of the herbivorous insect *Spodoptera littoralis*, (−)-usnic acid had a more dramatic effect on growth retardation and mortality, as reflected by an LD_{50} value 10 times lower than that of the (+)-enantiomer (Emmerich et al., 1993).

7. Toxicology

Data concerning usnic acid toxicity in humans are scarce. The only reported adverse effects are local irritation and allergic contact dermatitis, sometimes accompanied by conjunctivitis. Allergic contact dermatitis from lichens in general has long been recognized, but is considered relatively uncommon and the sensitizing potency of lichen constituents generally regarded as weak to moderate. Occupational contact dermatitis occurs mainly in forestry workers and wood cutters (cf. "woodcutter's eczema") coming into contact with lichens on the barks of trees, gardeners and those involved in harvesting or using lichens for floral decorations. Usnic acid has in many cases been considered partly responsible for such dermatological effects. Sensitivity can also develop on exposure to perfumes, after shave, deodorants, sunscreen products, cosmetics or antiseptic creams containing usnic acid and other lichen products. The most commonly used lichens in perfumery all contain usnic acid, i.e. *E. prunastri* (oak moss), *E. furfuracea* and *Ramalina* species. Both usnic acid enantiomers have been shown to give positive patch test reactions, but (−)-usnic acid was in one study shown to be more potent in guinea pig sensitization experiments (Hausen et al., 1993).

The only mammals consuming usnic-acid containing lichens in large quantities are reindeer and caribou which have specialized microorganisms in the rumen. Feeding experiments in other animals have shown usnic acid to be toxic in high doses. The development of ataxia in sheep and cattle, leading to paralysis of the extremities in severe cases, was attributed to usnic acid following consumption of the lichen *Parmelia mollius-cula* (Kingsbury, 1964). Sodium usneate administered i.v. to anaesthetized cats at doses of 10 mg/kg led to an augmented rate of metabolism, with symptoms such as

hyperventilation, increased oxygen consumption and rise in body temperature (Söderberg, 1953). Early toxicological data include i.v. LD_{50} doses determined as 25 mg/kg in mice, 30 mg/kg in rats and rabbits and 40 mg/kg in dogs (Virtanen and Kärki, 1956).

A commercial sample of usnic acid (enantiomer not specified) was not considered mutagenic in the Ames *Salmonella* assay (Shibamoto and Wei, 1984).

8. Synthesis of usnic acid and derivatives

Synthesis of (±)-usnic acid was achieved through phenolic oxidative coupling of methylphloracetophenone with potassium ferricyanide (Barton et al., 1956). The resulting dimer was acetylated to give (±)-usnic acid diacetate, which on hydrolysis afforded (±)-usnic acid.

The many functional groups of usnic acid make the molecule a good target for structural modification. The compound reacts with amines, hydrazines and acyl hydrazides to form condensation products, undergoes esterification, gives numerous degradation derivatives and forms dihydrousnic acid on reduction. Usnic acid salts are usually unstable. A large number of publications dating from 1962 to 1985 bear witness to the extensive work of Japanese researchers on the chemistry of usnic acid and reaction mechanisms (Takani and Takahashi, 1985, last in series). In another excellent series, Canadian scientists (Kutney et al., 1984, last in series) described preparation of numerous usnic acid biodegradation products and intermediate derivatives, as well as conversion of usnic acid to isousnic acid through base-catalyzed rearrangement.

Derivatization aimed at obtaining enhanced biological activity has often been attempted, and many such endeavours have been cited above in the relevant sections. Shibata et al. (1948) found that antitubercular activity decreased with acetylation of the two hydroxyls in ring A as well as with dihydro-usnic acid. In Finland derivatization was undertaken with the aim of overcoming poor solubility of usnic acid and enhancing the antimicrobial spectrum. The most promising results were attributed to product(s) obtained by reacting (−)-usnic acid with benzyl-dimethyl-(2-[2-(*p*-1,1,3,3-tetramethylbutyl-phenoxy)-ethoxy]ethyl)-ammonium hydroxide, a preparation which was subsequently marketed for various dermatological conditions, including bacterial and fungal infections (Virtanen and Kärki, 1956).

9. Bioproduction of usnic acid

Acquisition of natural lichen biomass for provision of secondary metabolites on a large scale is neither practical nor environmentally acceptable. Although the intricate physiological nature of the symbiosis makes cultivation of lichens difficult, progress in cultivation techniques for isolated symbionts and whole lichen thalli has been made, a lot of the success being attributable to Ahmadjian in Massachusetts and Yamamoto and co-workers in Japan (Kranner et al., 2002).

A method to initiate lichen tissue culture by using tiny segments of thalli was used to redifferentiate *Usnea* and *Ramalina* species (Yamamoto et al., 1985). Both cultures produced usnic acid, albeit in smaller concentration than observed in intact tissue. Productivity of the tissue cultures was however much higher since growth rate was considerably faster than the annual growth of intact thalli amounting to less than 1 cm. Usnic acid has been produced in substantial amounts by kaolinite-immobilized cells of *C. substellata* using acetate as precursor (Pereira et al., 1995).

10. Conclusive remarks

Despite a shortage of clinical trials, it can be concluded that scientific investigations justify to some extent reputed medicinal effects of lichens containing usnic acid. Antimycobacterial activity of usnic acid does not measure up to present day requirements for antitubercular drug development, but this does not rule out the possibility of usnic acid-containing lichens having been beneficial against tuberculosis.

Inhibitory activity of usnic acid against Gram positive- and anaerobic bacteria, including antibiotic-resistant pathogenic strains, definitely seems to merit further study. It should also be remembered that derivatization to potentiate antimicrobial activity has in some cases been successful despite a limited number of studies. Results obtained for other types of biological activity could also prove worthy of further pursuit, as could comparison of steric structure–activity relationships between the two enantiomers. Furthermore, usnic acid could serve as a lead structure for exploitation in novel therapeutic areas.

The mechanism of action expressed by usnic acid remains speculative. (+)-Usnic acid has been shown to be an uncoupler of oxidative phosphorylation in mouse-liver mitochondria at levels of 1 μM (Abo-Khatwa et al., 1996). Anti-inflammatory, analgesic and antipyretic effects have been suggested to be linked to inhibition of prostaglandin synthesis owing to the uncoupling effects on oxidative phosphorylation. Further studies are needed to clarify the mode of action of usnic acid on a molecular, therapeutic and toxicological basis.

In order for further studies to be feasible it is vital to find economical and ecologically acceptable ways of producing usnic acid. Further development of synthetic or tissue culture methods could prove valuable and advances in molecular biology will hopefully lead to acquisition potential through recombinant DNA techniques.

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