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Molecules of Interest

Hyperforin

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Abstract

Hyperforin is a polyprenylated acylphloroglucinol derivative from *Hypericum perforatum* (St. John's wort). It exhibits antidepressant activity by a novel mechanism of action, antibiotic activity against gram-positive bacteria, and antitumoral activity *in vivo*. However, it also produces drug–drug interactions by activation of the pregnan X receptor. No total synthesis has been described. Some natural and semisynthetic analogues are available to study structure–activity relationships. Enzymatically, the skeleton of hyperforin is formed by isobutyrophenone synthase from isobutyryl-CoA and three molecules of malonyl-CoA. The first prenylation step is catalyzed by a soluble and ion-dependent dimethylallyltransferase. Hyperforin mainly accumulates in pistils and fruits where it probably serves as defensive compound.

Keywords: Hyperforin; *Hypericum perforatum*; Clusiaceae; Structure; Pharmacology; Biosynthesis

1. Introduction

In 1975, Bystrov and co-workers isolated a complex compound from *Hypericum perforatum* L. (St. John's wort) (Fig. 1) and named it hyperforin (Bystrov et al., 1975). Today, *H. perforatum* is one of the best studied medicinal plants and hyperforin its best characterized constituent. Phytomedicines based on extracts from the plant's flowering upper parts are widely used as antidepressants (Müller, 2003; Butterweck, 2003). Their efficacy in mild to moderate depression was demonstrated in a number of clinical trials versus placebo and standard antidepressants (Whiskey et al., 2001). The relatively low rate of adverse effects and the good tolerability result in high patient acceptance. The detection of additional pharmacological activities in recent years further stimulated the interest in hyperforin (Medina et al., 2006).

2. Structure elucidation

Hyperforin is a bicyclic polyprenylated acylphloroglucinol derivative (Fig. 2). Its caged structure was determined by extensive chemical degradation and derivatisation, as well as by spectroscopic means (Bystrov et al., 1978 and literature cited therein). The relative stereochemistry was concluded from X-ray data of its 3,5-dinitrobenzoic acid ester and the absolute configuration was elucidated by single crystal X-ray analysis of its *p*-bromobenzoic acid ester (Brondz et al., 1982, 1983). Hyperforin is a mixture of interconverting tautomers, as indicated by the broad shape of most ^{1}H NMR signals and the poor resolution of many ^{13}C NMR lines (Verotta et al., 2000). When the tautomeric equilibrium of the enolized β-dicarbonyl system is covalently blocked, the derivatives show sharp NMR signals. All ^{1}H and ^{13}C NMR signals of hyperforin were unequivocally assigned by one- and two-dimensional NMR experiments (Adam et al., 2002). As a pure compound, hyperforin is poorly stable when exposed to light and oxygen (Maisenbacher and Kovar, 1992a; Erdelmeier, 1998; Liu et al., 2005). As a consequence, the compound was long neglected as a pharmacologically relevant constituent in

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Fig. 1. *Hypericum perforatum* L. (St. John's wort).

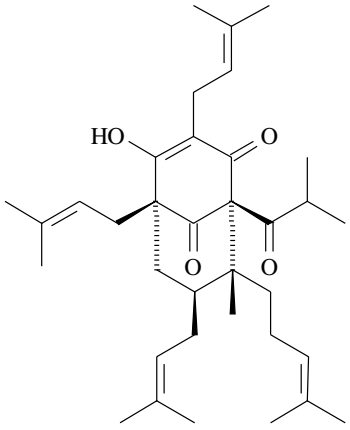

Fig. 2. Hyperforin.

commercial *H. perforatum* preparations (Chatterjee et al., 1998). Finally, the dicyclohexylammonium salt was found to be stable both at room temperature and under influence of air (Erdelmeier et al., 1999). The instability of hyperforin is due to the enolized β-dicarbonyl system because natural analogues lacking this moiety are stable (Verotta et al., 1999, 2000). The majority of commercial St. John's wort extracts prepared via aqueous alcoholic extraction contain 1–5% hyperforin (Lang et al., 2002).

3. Occurrence

The genus *Hypericum* (Clusiaceae = Guttiferae) encompasses about 450 species of trees, shrubs and herbs (Robson, 2003). *H. perforatum* is the only species to contain hyperforin as a quantitatively major constituent (Umek et al., 1999; Smelcerovic and Spiteller, 2006). In contrast to earlier studies (Berghöfer and Hölzl, 1986; Umek et al., 1999), nine *Hypericum* species have recently been found to have low hyperforin contents (*H. barbatum, H. richeri, H. rumeliacum, H. maculatum, H. tetrapterum, H. hirsutum, H. linarioides, H. olympicum*) (Smelcerovic and Spiteller, 2006). Furthermore, sepals of *H. elodes* contain hyperforin and adhyperforin (Piovan et al., 2004). In *H. perforatum*, the highest hyperforin concentrations were found in flowers and fruits. In the course of flower ontogenesis, the hyperforin content continuously increased from 2.5% in young buds (3–4 mm) to 8.5% in unripe fruits, the last developmental stage studied (Tekel'ová et al., 2000). This finding is in good agreement with the observation that hyperforin accumulates primarily in the pistil (Repčák and Mártonfi, 1997). In contrast, hypericins and flavonoids are mainly formed in sepals, petals, and stamens which fall off (Repčák and Mártonfi, 1997). In the flower of *H. calycinum*, the ovarian wall accumulates about 20% polyprenylated acyl- and benzoylphloroglucinols which act as defensive agents and protect the developing seeds against herbivores and microbes (Gronquist et al., 2001). During fruit ripening in *H. perforatum*, the content of the homologue adhyperforin increased approx. tenfold from 0.2% in flowers to 1.9% in capsules (Maisenbacher and Kovar, 1992b). In leaves, the hyperforin level was about 1.5% and did not appreciably change in response to feeding by a specialist beetle and generalists as well as mechanical wounding (Sirvent et al., 2003). Great intraspecific variation of the hyperforin content (0.3–1.3%) was observed with seedlings (Košuth et al., 2003). The hyperforin level in callus and cell cultures was about 0.15% (Kirakosyan et al., 2000), that of shoot cultures around 0.4% (Dias, 2003; Zobayed et al., 2003). Cell cultures of

H. calycinum formed mainly adhyperforin (Klingauf et al., 2004). Where the lipophilic hyperforins accumulate in intact plants and *in vitro* cultures at the tissue and subcellular levels remains open.

4. Derivatives

A limited number of natural and semisynthetic derivatives were obtained. The first natural analogue to be isolated was furohyperforin (orthoforin) which is present in the plant's aerial parts at a concentration of about 5% (Trifunović et al., 1998; Verotta et al., 1999; Orth et al., 1999). Later, another eight analogues were detected at low abundances (Verotta et al., 2000; Shan et al., 2001; Vajs et al., 2003). Whether these compounds are genuine constituents or artifacts of the extraction and isolation procedures is open. Chemical modification by acylation, alkylation, and oxidation led to a series of analogues which were used to study structure–activity relationships (Verotta et al., 2002, 2004). All these compounds were less potent inhibitors of synaptosomal serotonin reuptake than the parental compound, indicating a specific role for the enolized β-diketone moiety. The same was true with the natural analogues examined, suggesting that a covalent block of the tautomeric equilibrium by oxidation is detrimental for the pharmacological activity (Verotta et al., 1999, 2000; Orth et al., 1999). Furthermore, the activity of St. John's wort extracts appears to be strongly affected by oxidative degradation, unless hyperforin is stabilized as dicyclohexylammonium salt. A natural homologue of hyperforin is adhyperforin where the isopropyl ketone side chain is replaced by a 2-methyl propyl ketone substituent (Maisenbacher and Kovar, 1992b). In St. John's wort extracts, the hyperforin to adhyperforin ratio is 5–10 to 1 (Lang et al., 2002). The reuptake inhibitory activity of adhyperforin is comparable to that of hyperforin (Jensen et al., 2001). An interesting hyperforin-cadinane adduct is hydroperoxycadiforin which bears a hydroperoxyl group on the sesquiterpene moiety and was isolated from stems and leaves (Rücker et al., 1995).

5. Pharmacological activities

5.1. Reuptake inhibition

Hyperforin is a broad-band neurotransmitter reuptake inhibitor which affects the synaptosomal uptake of serotonin, dopamine, noradrenalin (norepinephrine), glutamate and gamma-aminobutyric acid (GABA) with similar efficiencies (Müller, 2003). This acute effect is followed by adaptive changes in the receptor system. The underlying mode of action is unique to hyperforin because the compound does not interact directly with the transmitter transporters but elevates the intracellular sodium concentration, thereby inhibiting the gradient-driven neurotransmitter reuptake (Singer et al., 1999). This effect on $[Na^+]_i$ has been attributed to the activation of nonselective cation channels (Treiber et al., 2005). In contrast, synthetic antidepressants are competitive inhibitors of either one or maximally two transporters at the transmitter binding sites. Thus, hyperforin is not only structurally but also functionally a new antidepressant. Besides hyperforin, hypericins and flavonoids are discussed to contribute to the antidepressant activity of St. John's wort by different mechanisms of action (Butterweck, 2003).

5.2. Antibacterial potency

A long known property of hyperforin is its antibacterial activity (Gurevich et al., 1971). Hyperforin inhibited the growth of gram-positive bacteria such as *Corynebacterium diphtheriae* at concentrations as low as 0.1 μg/ml (Schempp et al., 1999). Multiresistant *Staphylococcus aureus* strains were also susceptible to hyperforin (Schempp et al., 1999; Reichling et al., 2001). No growth inhibition was observed with gram-negative bacteria and *Candida albicans*. The antibiotic potential of hyperforin may explain the traditional use of St. John's wort preparations for the local treatment of infected wounds (Schempp et al., 1999). In addition, the pronounced antiinflammatory activity of hyperforin may provide a rationale for the topical treatment of inflammatory skin disorders (Schempp et al., 2000; Albert et al., 2002).

5.3. Antitumoral properties

Hyperforin is a promising novel anticancer agent. It inhibited the growth of a wide range of human and rat tumor cell lines by induction of apoptosis in a dose-dependent manner with IC_{50} values of 3–20 μM (Schempp et al., 2002; Hostanska et al., 2003). The compound was also active in an animal model. Its antiproliferative effect *in vivo* was comparable to that exerted by paclitaxel, and that in the absence of any signs of acute toxicity (Schempp et al., 2002). Hyperforin prevents and contrasts cancer spread and metastatic growth (Donà et al., 2004) and inhibits angiogenesis *in vivo* and several key steps of this process *in vitro* (Martínez-Poveda et al., 2005). A synthetic hyperforin derivative with improved stability and solubility properties was found to retain *in vitro* and *in vivo* the antitumor properties of the parental compound without inducing toxicity (Gartner et al., 2005).

5.4. Cytochrome P450 and P-glycoprotein induction

Hyperforin is also a potent ligand for the pregnane X receptor (PXR) (Moore et al., 2000; Cantoni et al., 2003). The crystal structure of hyperforin in complex with the ligand binding domain of human PXR was described (Watkins et al., 2003). The PXR ligand binding cavity is able to expand and contract depending on the character of the bound ligand. Among the genes regulated by PXR is the

gene encoding CYP3A4. This monooxygenase is involved in hepatic drug metabolism of >50% of all drugs, leading to some severe drug–drug interactions (Moore et al., 2000). Co-medication with St. John's wort extract can lead to a dramatic reduction of the plasma level of the co-administered drug and thereby its clinical efficacy (Madabushi et al., 2006). In case of immunosuppressants, HIV protease inhibitors, and cancer drugs, the concurrent use can even have life-threatening consequences and is a clear contraindication (Ruschitzka et al., 2000; Piscitelli et al., 2000; Mathijssen et al., 2002). Hyperforin also increases the expression of the intestinal multidrug transporter P-glycoprotein encoded by the MDR1 gene (Gutmann et al., 2006). This transmembrane efflux pump is a member of the ABC transporter superfamily and transports drugs out of the epithelial cell into the intestinal lumen (Wang et al., 2001). Thus, hyperforin contributes to both the therapeutic and the adverse effects of St. John's wort preparations.

6. Synthesis

Hyperforin has a unique molecular architecture. Despite its relatively small size, the structure constitutes a thorny synthetic challenge and remains to this day defiant to chemical synthesis (Nicolaou et al., 2005). It contains asymmetric vicinal quaternary centers and a densely functionalized tetracarbonyl array. Its prenylated bicyclo[3.3.1]nonanone core is conserved among a number of other acylphloroglucinol derivatives. Synthetic routes to such polycyclic polyprenylated acylphloroglucinols have been developed (Usuda et al., 2002; Spessard and Stoltz, 2002; Kraus et al., 2003; Young and Zeng, 2002; Ciochina and Grossman, 2003). Recently, a novel synthetic sequence to polyfunctional, bridged medium-sized rings from simple cyclic ketones has been reported (Nicolaou et al., 2005). To date, however, no total synthesis has been described for any of the more than 50 members of this class of substances.

7. Biosynthesis

Enzymatic hyperforin formation is poorly understood. Feeding of ^{13}C-labeled glucose to *H. perforatum* sprouts and subsequent analysis of the isolated hyperforin by quantitative NMR spectroscopy demonstrated that the acylphloroglucinol nucleus is generated via a polyketide mechanism with isobutyryl-CoA as starter molecule, itself being derived from valine (Adam et al., 2002) (Fig. 3). The five isoprenoid moieties involved are derived predominantly (>98%) via the non-mevalonate (MEP) pathway. Triple electrophilic substitution of the unsubstituted hyperforin nucleus involves two dimethylallyl diphosphate (DMAPP) units and one geranyl diphosphate (GPP) molecule. The ring closure to give the bicyclic system is triggered by electrophilic attack of a third DMAPP on the 2′/3′

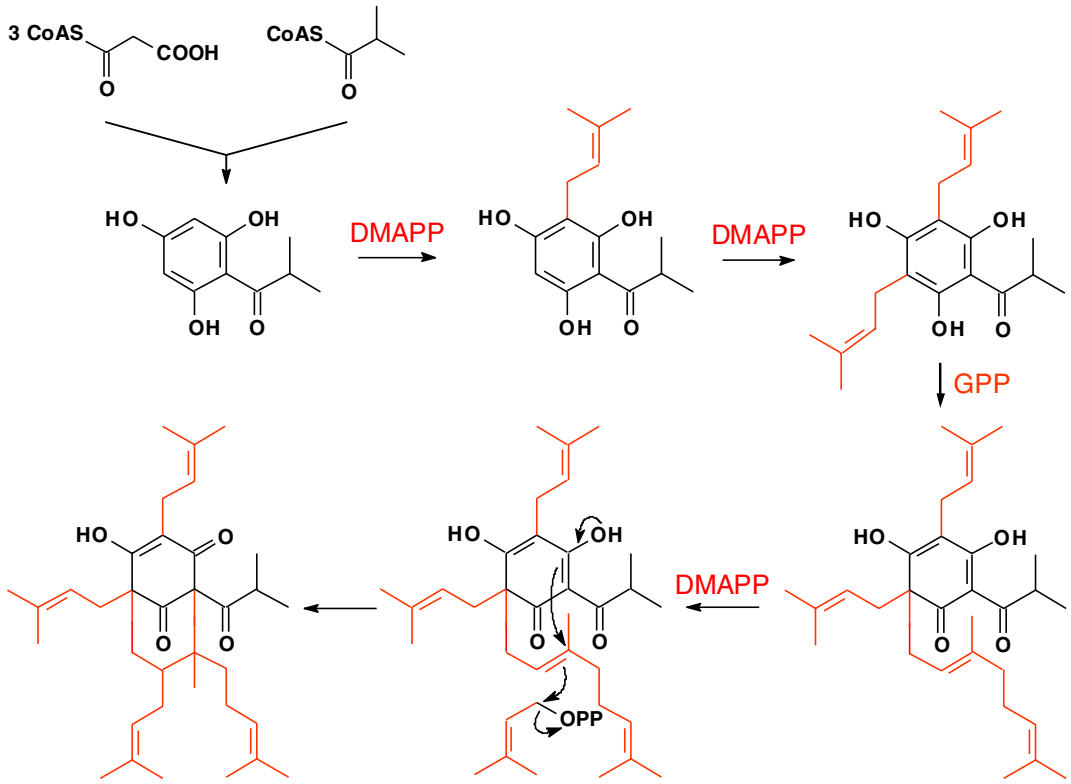

Fig. 3. Proposed hyperforin biosynthetic pathway.

double bond of the preimplanted geranyl chain. The sequence of the steps, however, is largely open.

Formation of the hyperforin nucleus was detected in cell-free extracts from *H. calycinum* cell cultures (Klingauf et al., 2004). Isobutyrophenone synthase (BUS) catalyzes the condensation of isobutyryl-CoA with three molecules of malonyl-CoA to give a linear tetraketide intermediate which is cyclized by intramolecular Claisen condensation to yield phlorisobutyrophenone. BUS was separated by anion exchange chromatography from two other type III polyketide synthases. Benzophenone synthase (BPS) prefers benzoyl-CoA as starter molecule and catalyzes the formation of phlorbenzophenone, whereas chalcone synthase (CHS) prefers 4-coumaroyl-CoA and forms naringenin chalcone (Liu et al., 2003). Both products can undergo intramolecular cyclization to give xanthones and flavonoids, respectively. Since the acetate–malonate pathway probably also underlies the formation of hypericins (Bais et al., 2003), polyketide metabolism plays a central role in the biosynthesis of the active *H. perforatum* constituents. cDNAs encoding BPS and CHS were cloned from *H. androsaemum* cell cultures (Liu et al., 2003) and a gene encoding a BUS-like isovalerophenone synthase was cloned from hop (*Humulus lupulus*) (Okada and Ito, 2001).

The first prenylation step was detected in *H. calycinum* cell cultures (Boubakir et al., 2004). During cell culture growth, the formation of hyperforins was preceded by increases in BUS and prenyltransferase activities. The aromatic prenyltransferase preferred DMAPP as prenyl donor and phlorisobutyrophenone as prenyl acceptor. The enzyme was soluble and dependent on a divalent cation, with Fe^{2+} being the most efficient cofactor. Prenyltransferases with similar properties participate in the biosynthesis of bitter acids in hop (*Humulus lupulus*) (Zuurbier et al., 1998) and cannabinoids in hemp (*Cannabis sativa*) (Fellermeier and Zenk, 1998). However, the majority of aromatic prenyltransferases are integral membrane proteins and contain a typical prenyl diphosphate binding site [(N/D)DXXD] (Pojer et al., 2003). Another class of transferases includes soluble enzymes which in addition lack the prenyl diphosphate binding site and thereby the absolute requirement for a divalent cation (Tsai et al., 1995; Pojer et al., 2003).

8. Perspectives

Hyperforin is an attractive compound for biotechnological research because it combines intriguing pharmacological activities and defiance to chemical synthesis. Every effort has to be directed at elucidating the biosynthesis of hyperforin with respect to the enzymes and genes involved and the temporal and spatial regulation. This information, together with an efficient transformation system, lays the foundation for metabolic engineering of hyperforin biosynthesis. Hyperforin is an interesting lead compound because it differs structurally from all known antidepressants, antibiotics, and antitumorals. It may be possible to obtain novel hyperforin analogues which retain the pharmacological activity but fail to provoke drug–drug interactions by PXR binding.

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