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The first prenylation step in hyperforin biosynthesis

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Abstract

Prenylation reactions contribute considerably to the diversity of natural products. Polyprenylated secondary metabolites include hyperforin which is both quantitatively and pharmacologically a major constituent of the medicinal plant *Hypericum perforatum* (St. John's wort). Cell cultures of the related species *Hypericum calycinum* were found to contain a prenyltransferase activity which is likely to catalyze the first prenylation step in hyperforin biosynthesis. The enzyme was soluble and dependent on a divalent cation, with Fe^{2+} leading to maximum activity (K_m = 3.8 mM). The preferred prenyl donor was DMAPP (K_m = 0.46 mM) and the preferred prenyl acceptor was phlorisobutyrophenone (K_m = 0.52 mM). A broad pH optimum from 6.5 to 8.5 and a temperature optimum from 35 to 40 °C were observed. The formation of hyperforins in *H. calycinum* cell cultures was preceded by an increase in dimethylallyltransferase activity, with the maximum specific activity being 3.6 μkat/kg protein.

Keywords: *Hypericum calycinum* cell cultures; Hyperforin biosynthesis; Prenylation; Phlorisobutyrophenone dimethylallyltransferase

1. Introduction

Many natural products contain terpenoid residues. Carbon skeletons derived for example from the shikimate and polyketide pathways can be modified by attaching isoprenoid chains. A particularly common terpenoid group is the dimethylallyl substituent. This residue is able to cyclize with an *ortho* hydroxyl group, leading to the formation of either five-membered furan or six-membered pyran heterocycles (Stanjek et al., 1999). These rings are commonly encountered in natural products, such as coumarins and flavonoids. Cyclizations can also result from formation of carbon–carbon bonds, giving complex molecules with caged skeletons. These bridged polycyclic systems are frequently found in phloroglucinol derivatives, such as prenylated benzophenones (Hu and Sim, 2000) and hyperforins.

Hyperforin (Fig. 1) is a secondary metabolite with interesting pharmacological properties. It contributes to the antidepressant activity of St. John's wort (*Hypericum perforatum*) extracts by inhibiting non-selectively the synaptosomal reuptake of a number of neurotransmitters (Müller, 2003). The underlying mode of action is unique to hyperforin which does not directly interact with the transmitter transporters but elevates the intracellular sodium concentration, thereby inhibiting the gradient-driven neurotransmitter uptake. However, hyperforin also binds to the pregnane X receptor which regulates the expression of CYP 3A4 (Moore et al., 2000; Cantoni et al., 2003). The

Abbreviations: DMAPP, dimethylallyl diphosphate; IPP, isopentenyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate.

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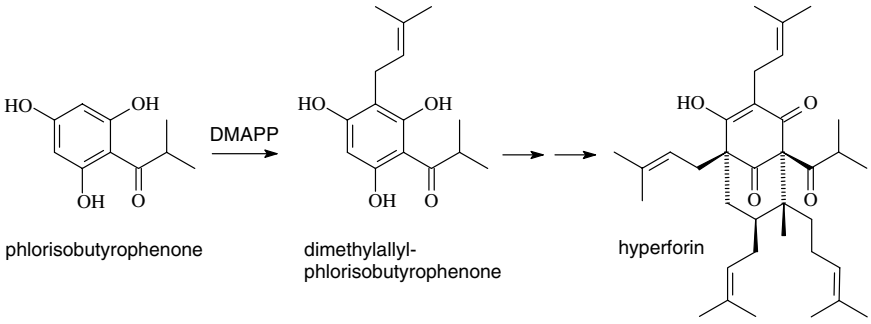

Fig. 1. Prenylation of the hyperforin nucleus by phlorisobutyrophenone dimethylallyltransferase.

compound thus contributes to both the therapeutic and the side effects of St. John's wort preparations. Hyperforin also exhibits antibiotic properties (Schempp et al., 1999) and inhibits in vivo the growth of human and rat tumour cell lines by induction of apoptosis (Schempp et al., 2002).

Little is known about the biosynthesis of hyperforin. Recently, the formation of hyperforin and its homologue adhyperforin was observed in cell cultures of *Hypericum calycinum* which are thus a valuable in vitro system for studying hyperforin biosynthesis (Klingauf et al., 2005). The cultured cells contained, besides chalcone synthase and benzophenone synthase, isobutyrophenone synthase which catalyzes the sequential condensation of one molecule of isobutyryl-CoA with three molecules of malonyl-CoA to form the hyperforin nucleus (Klingauf et al., 2005). This aromatic intermediate undergoes a series of prenylation reactions. Here we report the first prenylation step.

2. Results

2.1. Detection of prenyltransferase activity

Phlorisobutyrophenone and DMAPP were chemically synthesized and incubated with cell-free extracts from 3-day-old *H. calycinum* cell cultures in the presence of a divalent cation. These incubations resulted in the formation of an enzymatic product, as demonstrated by HPLC and GC analyses (Fig. 2). No prenyltransferase activity could be detected when boiled protein extract was used and either phlorisobutyrophenone or DMAPP was omitted. The enzymatic product was extracted from large-scale incubations, purified by TLC and analyzed by GC–MS. In comparison with a sample of chemically synthesized reference compound, it was identified as dimethylallylphlorisobutyrophenone. The synthetic compound and the enzymatic product exhibited identical retention times and mass spectra. This was also true for their acetylated derivatives (Fig. 2).

2.2. Substrate specificity and kinetic data

The transferase used DMAPP as prenyl donor (Table 1). Product formation also occurred in the presence of IPP, which, however, was attributed to IPP isomerase activity in the crude extract. No enzyme activity was observed with GPP and FPP as prenyl donors. The preferred prenyl acceptor was phlorisobutyrophenone (Table 1). High transferase activity was also found with phloracetophenone. The product resulting from this acceptor was identified as dimethylallylphloracetophenone by HPLC and GC–MS in comparison with a sample of chemically synthesized reference compound. No enzyme activity occurred with phloroglucinol as prenyl acceptor. Prenylation of phlorisobutyrophenone followed Michaelis–Menten kinetics. The K_m values were 0.52 mM for the acceptor and 0.46 mM for DMAPP, as calculated from Lineweaver–Burk plots (Fig. 3). The formation of hyperforins in *H. calycinum* cell cultures occurred between day 2 and 6 (Klingauf et al., 2005) and was preceded by an increase in dimethylallyltransferase activity (Fig. 4). The maximum specific enzyme activity was 3.6 μkat/kg protein.

2.3. Metal ion dependence, solubility and pH and temperature optima

Dimethylallyltransferase activity was strictly dependent on a divalent cation, with Fe^{2+} being the most efficient cofactor (Fig. 5). This finding was not due to the reducing properties of Fe^{2+} because experiments in the presence and in the absence of 10 mM ascorbic acid gave the same result. The K_m for Fe^{2+} was 3.8 mM (Fig. 3). The prenyltransferase showed a broad pH optimum from 6.5 to 8.5, which has previously been observed with other prenyltransferases (Yamamoto et al., 1997). Under the assay conditions described, optimal product formation was observed at 35–40 °C. The reaction was linear with time up to 30 min and with the protein amount up to 80 μg in the assay. After ultracentrifugation, about 80% of the enzyme activity was found in the supernatant, indicating a soluble dimethylallyltransferase (Table 2).

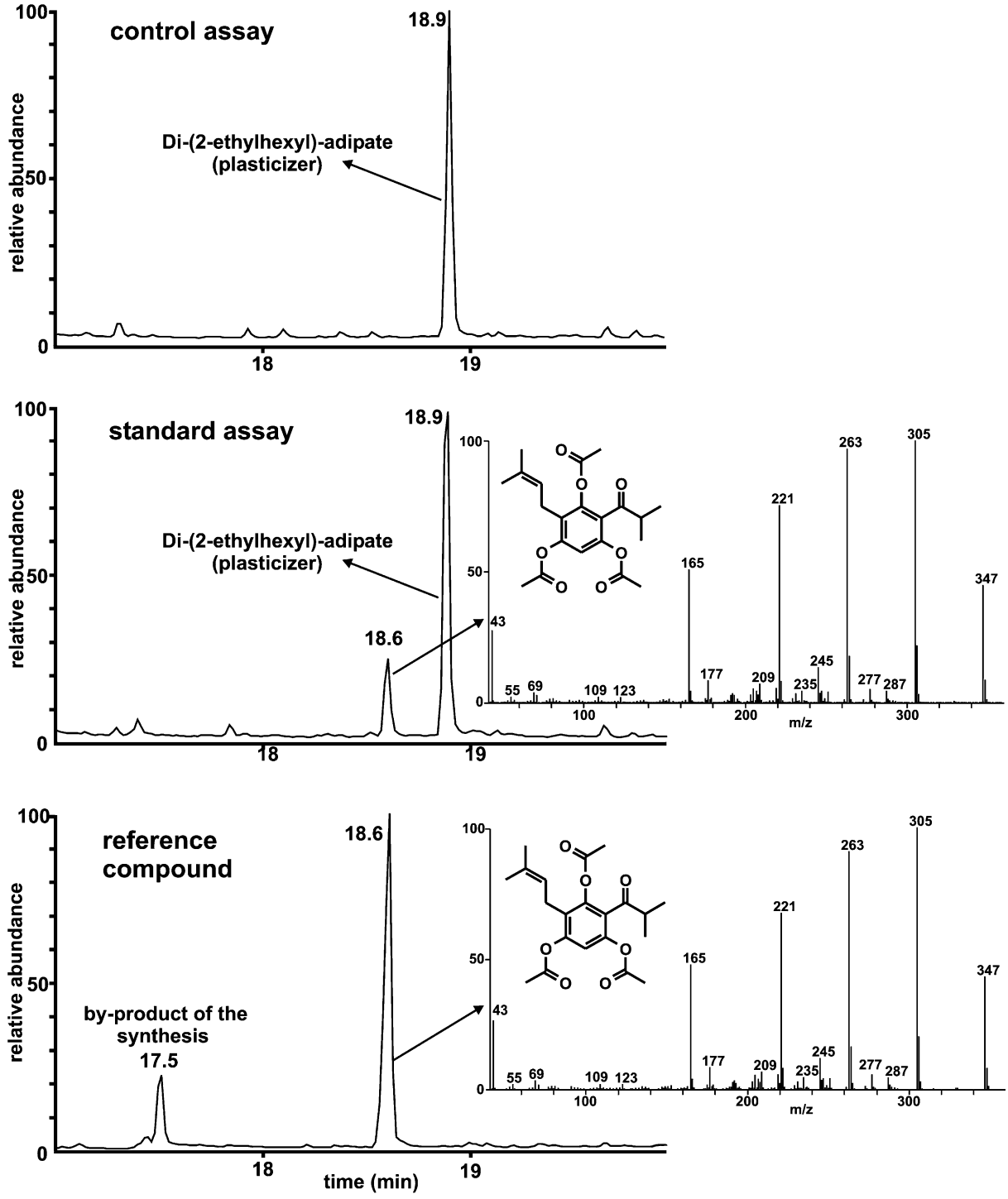

Fig. 2. GC–MS analysis of prenyltransferase assays. The control assay contained heat-denatured protein. The enzymatic product and the synthetic reference compound were acetylated.

3. Discussion

Prenylation reactions contribute considerably to the diversification of secondary metabolites. Prenyltransferases participate in the biosynthesis of flavonoids (Yamamoto et al., 2000), coumarins (Hamerski et al., 1990), cannabinoids (Fellermeier and Zenk, 1998), hop bitter acids (Zuurbier et al., 1998), and shikonin (Yazaki et al., 2002). The shikonin-related enzyme was the first prenyltransferase to be cloned from a plant source, namely cell cultures of *Lithospermum erythrorhizon*. The enzyme catalyzes the transfer of a geranyl residue to 4-hydroxybenzoic acid.

Table 1
Substrate specificity of phlorisobutyrophenone dimethylallyltransferase from *H. calycinum* cell cultures

Prenyl donor	Rel. enzyme activity (%)	Prenyl acceptor	Rel. enzyme activity (%)
Dimethylallyl diphosphate (DMAPP)	100[a]	Phlorisobutyrophenone	100[a]
Isopentenyl diphosphate (IPP)	30	Phloracetophenone	80
Geranyl diphosphate (GPP)	<0.3[b]	Phloroglucinol	<0.3[b]
Farnesyl diphosphate (FPP)	<0.3[b]		
Geranylgeranyl diphosphate (GGPP)	<0.3[b]		

[a] Specific activity: 3.6 μkat/kg.
[b] Detection limit: 1 ng.

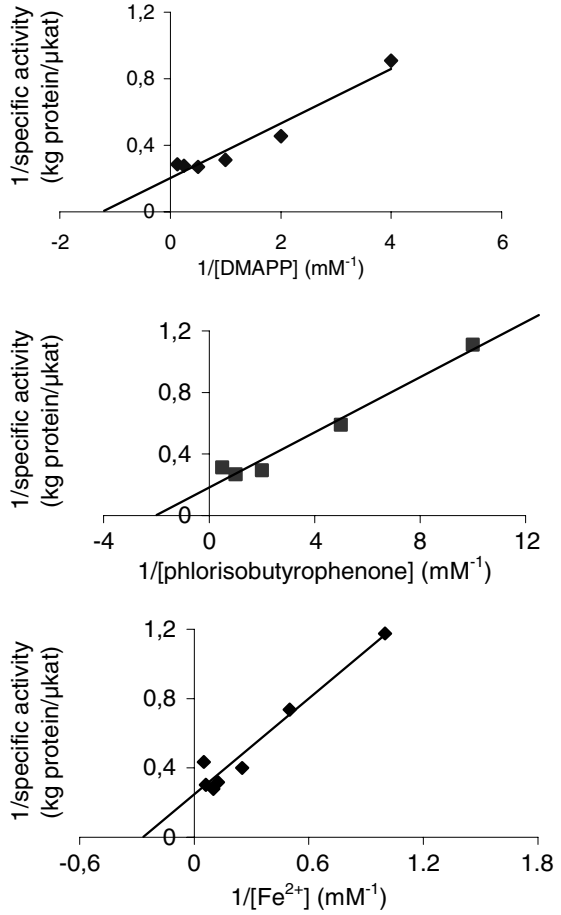

Fig. 3. Dependence of dimethylallyltransferase activity on the concentrations of phlorisobutyrophenone, DMAPP, and Fe^{2+}.

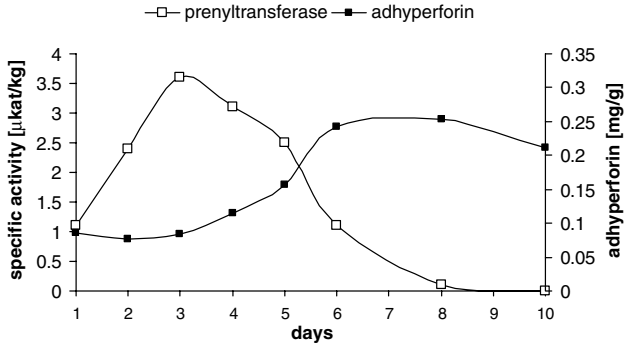

Fig. 4. Adhyperforin formation (data from Klingauf et al., 2005) and changes in prenyltransferase activity during cell culture growth. Values are means of two experiments.

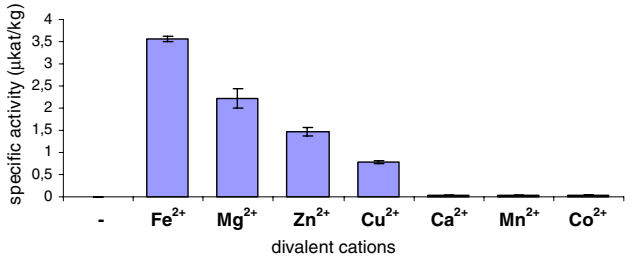

Fig. 5. Effect of divalent cations on dimethylallyltransferase activity. Bars represent means and standard deviations from three determinations.

In this study, we detected and characterized the dimethylallyltransferase which is likely to catalyze the first prenylation step in hyperforin biosynthesis. However, it cannot yet be fully excluded that the enzyme might be related to the formation of other prenylated secondary metabolites, such as flavonoids and benzophenones. The enzyme is a so-called aromatic prenyltransferase which catalyzes the formation of a carbon–carbon bond between an aromatic nucleus and a prenyl group. The majority of the aromatic prenyltransferases are integral membrane proteins and their catalytic cavities include a typical prenyl disphophate binding site [(N/D)DXXD] (Pojer et al., 2003). This binding motif is also present in the chain-elongating *trans*-prenyltransferases, such as geranyl diphosphate synthase, farnesyl diphosphate synthase, and geranylgeranyl diphosphate synthase (Wang and Ohnuma, 1999). The cloned geranyltransferase from *L. erythrorhizon* also belongs to this group (Yazaki et al., 2002).

Another class of aromatic prenyltransferases includes soluble enzymes which, in addition, lack the characteristic prenyl diphosphate binding site and thus the absolute requirement for a divalent cation. Examples are the microbial enzymes 4-dimethylallyltryptophan synthase and CloQ which are involved in ergot alkaloid and clorobiocin biosyntheses, respectively (Tsai et al., 1995; Pojer et al., 2003).

Table 2
Prenyltransferase activity in cell fractions after ultracentrifugation

Cell fraction	Protein (mg)	Total activity		Specific activity (μkat/kg)
		pkat	%	
Cell-free extract	5.5	19.6	100	3.6
Supernatant (100,000*g*)	4.9	15.7	80.1	3.2
Pellet (100,000*g*)	0.5	0.07	0.4	0.1

The dimethylallyltransferase from cultured *H. calycinum* cells differs from the members of the above-mentioned classes of prenyltransferases in that it is soluble and its activity dependent on a divalent cation. It is the first plant prenyltransferase to prefer Fe^{2+}. Other enzymes are most active with Mg^{2+} or Mn^{2+} (Hamerski et al., 1990; Laflamme et al., 1993; Zuurbier et al., 1998). Prenyltransferases with similar properties, i.e. soluble and ion-dependent, are involved in the biosynthesis of bitter acids in hop (*Humulus lupulus*; Zuurbier et al., 1998) and cannabinoids in hemp (*Cannabis sativa*; Fellermeier and Zenk, 1998). In glandular hairs of hop cones, the enzyme activity catalyzes the stepwise prenylation of acylphloroglucinols to yield deoxyhumulone and deoxycohumulone which are the direct precursors of the hop bitter acids. In leaves of Indian hemp, the enzyme catalyzes the alkylation of olivetolic acid with GPP. It will be interesting to study the evolutionary relationship between the dimethylallyltransferase from *H. calycinum* cell cultures and the enzymes of the two other classes of prenyltransferases.

4. Experimental

4.1. Chemicals

Phlorisobutyrophenone was synthesized as described previously (Klingauf et al., 2005). Phloracetophenone and 3,3-dimethylallyl bromide were purchased from Acros Organics (Geel, Belgium) and Fluka (Buchs, Switzerland), respectively. 3,3-Dimethylallyl alcohol and trichloroacetonitrile were obtained from Aldrich (Steinheim, Germany).

4.2. Synthesis of dimethylallylphlorisobutyrophenone (3-(3-methyl-2-butenyl)-2,4,6-trihydroxyisobutyrophenone)

The reaction was conducted according to the method of Xiao et al. (1998). 2,4,6-Trihydroxyisobutyrophenone (4.1 mmol) was dissolved in 10 ml aq. KOH (10%) and cooled to 0 °C. Prenyl bromide (6.1 mmol) was added dropwise over 5 min. After stirring for 1 h at room temperature, the reaction mixture was poured into 10 ml of ice water, acidified with HCl (10%) to pH 2 and extracted with ethyl acetate (3 × 10 ml). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. Due to instability in silica gel, the product was chromatographed on Sephadex LH-20 (2.5 × 40 cm; Amersham Biosciences, Freiburg, Germany) using methanol (70%) as solvent. Further purification was achieved by semi-preparative HPLC (0.8 × 25 cm; Eurosorb-100 C_{18}) using water (A) and methanol (B) for the following gradient: 50% B for 2 min, 50–80% B in 30 min, 80–100% B in 5 min. The flow rate was 3 ml/min and the detection wavelength 290 nm. The spectroscopic properties of the product agreed with the literature (Kuhnke and Bohlmann, 1985; Fung et al., 1994). For acetylation, the product was dissolved in 10 μl dry pyridine and 10 μl acetic acid anhydride. After 1 h at room temperature, the reaction was quenched by addition of 500 μl methanol and an aliquot was analyzed by GC–MS.

3-(3-Methyl-2-butenyl)-2,4,6-trihydroxyisobutyrophenone: R_f (silica gel, diethyl ether:pentane 60:40) 0.75; RI (ZB1/ZB5) 2223/2274; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (6H, *d*, J = 7 Hz, CH_3×2), 1.78 (3 H, *d*, J = 1 Hz, CH_3), 1.82 (3 H, *s*, CH_3), 3.36 (2 H, *d*, J = 7.2 Hz, =CH–CH_2), 3.8 (1H, *qq*, –CH–$(CH_3)_2$), 5.25 (1H, *m*, =CH) 5.8 (1 H, *s*, $C_{arom.}H$); EIMS, 70 eV, *m*/*z* (rel. int.): 264 (18, $[M]^+$), 221 (39), 209 (6), 191 (3), 177 (3), 165 (100), 153 (5), 69 (5), 55 (3), 43 (3).

3-(3-Methyl-2-butenyl)-2,4,6-triacetoxyisobutyrophenone: RI (ZB1) 2341; EIMS, 70 eV, *m*/*z* (rel. int.): 347 (27, $[M–C_3H_7]^+$), 305 (72, $[M–C_3H_7–1×ketene]^+$), 277(4) 263 (86, $[M–C_3H_7–2×ketene]^+$), 245 (13), 221(100, $[M–C_3H_7–3×ketene]^+$), 207 (6),177 (12), 165 (81), 43 (40).

4.3. Synthesis of dimethylallylphloracetophenone (3-(3-methyl-2-butenyl)-2,4,6-trihydroxyacetophenone)

We used the procedure described above, except that 2,4,6-trihydroxyacetophenone (4 mmol) was dissolved in 5.3 ml aq. KOH (10%) and 10.5 mmol of prenyl bromide were added. The product was purified on a silica gel column (2.5 × 40 cm) using diethyl ether:pentane 60:40 as solvent. The spectroscopic properties of the product were consistent with published data (Jakupovic et al., 1986). The acetylation procedure is described under Section 4.2.

3-(3-Methyl-2-butenyl)-2,4,6-trihydroxyacetophenone: R_f (silica gel, diethyl ether:pentane 60:40) 0.44; RI (ZB5) 2177; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.78 (3 H, *s*, CH_3), 1.83 (3 H, *s*, CH_3), 2.66 (3 H, *s*, COCH_3), 3.36 (2 H, *d*,

J = 7.2 Hz, CH–CH_2), 5.25 (1H, *m*, =C*H*–CH2), 5.83 (1 H, *s*, $C_{arom.}H$); EIMS, 70 eV, *m/z* (rel. int.): 181 (100, $[M–C_4H_7]^+$), 236 (88, $[M]^+$), 165 (34), 221 (32, $[M–CH_3]^+$), 193 (32, $[M–CH_3CO]^+$), 163 (20), 43 (18), 153 (14), 203 (11), 182 (9), 69 (9),168 (7).

3-(3-Methyl-2-butenyl)-2,4,6-triacetoxyacetophenone: RI (ZB1) 2248; EIMS, 70 eV, *m/z* (rel. int.): 319 (40, [M –CH_3CO], 277 (96, [M–CH_3CO–1×keten]), 278 (19), 235 (100, [M–CH_3CO–2×keten]), 236 (34), 223 (10), 221 (13) 193 (17, [M–CH_3CO–3×keten]), 181 (53), 168 (4), 165 (8), 163 (4), 109 (3), 43 (35).

4.4. Synthesis of DMAPP

DMAPP was prepared as described by Cornforth and Popjak (1969), starting with 3,3-dimethylallyl alcohol (6 mmol), trichloroacetonitrile (36 mmol) and di-triethylammonium phosphate (14.4 mmol). The reaction product was purified on a silica gel 60 (Merck, Darmstadt, Germany) column (2.5 × 40 cm) using *n*-propanol:ammonia:water (6:3:1) as solvent and lyophilized. The NMR spectra of the product agreed with published data (Davisson et al., 1986).

R_f (silica gel, *n*-propanol:ammonia:water 6:3:1) 0.31; ^{1}H NMR (400 MHz, D_2O) δ 1.71 (3 H, *s*, CH_3), 1.75 (3 H, *s*, CH_3), 4.44 (2 H, *dd*, $J_{H,H}$ = 7 Hz, $J_{H,P}$ = 7 Hz, CH_2), 5.44 (1 H, *t*, $J_{H,H}$ = 7 Hz, $(CH_3)_2$–C*H*); ^{13}C NMR (100 MHz, D_2O) δ 19.8, 27.5, 65,2 (*d*, $J_{C,P}$ = 5.3 Hz), 122.3 (*d*, $J_{C,P}$ = 8.6 Hz), 142.5; ^{31}P NMR (160 MHz, D_2O) δ −9.7 (1 P, *d*, $J_{P,P}$ = 21.6 Hz), −6.9 (1 P, *d*, $J_{P,P}$ = 21.6 Hz).

4.5. Cell cultures

Cell suspension cultures were grown as described previously (Klingauf et al., 2005).

4.6. Enzyme preparation and ultracentrifugation

The procedure was carried out at 0–4 °C. Three-day-old cultured cells (5 g) were collected by suction filtration, mixed with 0.5 g Polyclar AT (Serva, Heidelberg, Germany) and homogenized in 2.5 ml of 0.1 M sodium phosphate buffer, pH 6.5 containing 1 mM DTT, using a Teflon homogenizer. The homogenate was centrifuged at 10,000*g* for 15 min and the supernatant either passed through a PD-10 column (Amersham Biosciences) equilibrated with 0.1 M Tris–HCl pH 7.5 containing 1 mM DTT or subjected to ultracentrifugation at 100,000*g* for 30 min. Protein concentrations were assayed by the method of Bradford (1976).

4.7. Transferase assay and kinetic studies

The standard assay (250 µl) contained 2 mM phlorisobutyrophenone, 2 mM DMAPP, 10 mM $FeCl_2$, 0.1 M Tris–HCl, pH 7.5 and 50 µl desalted protein extract (approx. 100 µg protein). After incubation for 30 min at 37 °C, the enzymatic products were extracted twice with ethyl acetate. The combined organic phases were evaporated to dryness and the residue was dissolved in 50 µl methanol. For large-scale incubations the assay volume was increased to 4 ml.

K_m values were calculated from Lineweaver–Burk plots. For DMAPP, the concentration was changed between 0.2 and 16 mM while that of phlorisobutyrophenone was kept constant at 2 mM. For phlorisobutyrophenone, the concentration varied between 0.1 and 8 mM while the assays contained 2 mM DMAPP. Two independent experiments were performed and mean values calculated.

4.8. Analytical methods

HPLC of enzymatic products. We used a 1525 Binary HPLC Pump coupled with a 2487 Dual Absorbance Detector and equipped with the Breeze software 3.20 (Waters, Eschborn, Germany). The mobile phase consisted of water (A) and methanol (B) on a C_{18} 100-5 column (0.4 × 25 cm; Macherey-Nagel, Düren, Germany). The gradient was as described under Section 4.2. The flow rate was 1 ml/min and the detection wavelength 290 nm.

Isolation of enzymatic products. Enzyme assays (4 ml) were extracted with ethyl acetate (3 × 6 ml). After removal of the combined organic phase in vacuo, the residue was dissolved in methanol and separated by TLC (silica gel 60 F_{254} (Merck), diethyl ether:pentane 60:40). The zones with R_f 0.75 (dimethylallyl phlorisobutyrophenone) and 0.44 (dimethylallyl phloracetophenone) were scraped off. The compounds were eluted with ethyl acetate, acetylated as described under Section 4.2 and analyzed by GC–MS.

GC–MS. Analyses were performed as described previously (Liu et al., 2004).

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References

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Cantoni, L., Rozio, M., Mangolini, A., Caccia, S., 2003. Hyperforin contributes to the hepatic CYP3A-inducing effect of *Hypericum perforatum* extract in the mouse. Toxicol. Sci. 75, 25–30.

Cornforth, R.H., Popjak, G., 1969. Chemical synthesis of substrates of sterol biosynthesis. Meth. Enzymol. 15, 359–390.

Davisson, V.J., Woodside, A.B., Neal, T.R., Stremler, K.E., Muehlbacher, M., Poulter, C.D., 1986. Phosphorylation of isoprenoid alcohols. J. Org. Chem. 51, 4768–4779.

Fellermeier, M., Zenk, M.H., 1998. Prenylation of olivetolate by a hemp transferase yields cannabigerolic acid, the precursor of tetrahydrocannabinol. FEBS Lett. 427, 283–285.

Fung, S.Y., Brussee, J., van der Hoeven, R.A.M., Niessen, W.M.A., Scheffer, J.J.C., Verpoorte, R., 1994. Analysis of proposed aromatic precursors of hop bitter acids. J. Nat. Prod. 57, 452–459.

Hamerski, D., Schmitt, D., Matern, U., 1990. Induction of two prenyltransferases for the accumulation of coumarin phytoalexins in elicitor-treated *Ammi majus* cell suspension cultures. Phytochemistry 29, 1131–1135.

Hu, L.H., Sim, K.Y., 2000. Sampsoniones A–M, a unique family of caged polyprenylated benzoylphloroglucinol derivatives, from *Hypericum sampsonii*. Tetrahedron 56, 1379–1386.

Jakupovic, J., Kuhnke, J., Schuster, A., Metwally, M.A., Bohlmann, F., 1986. Phloroglucinol derivatives and other constituents from South African *Helichrysum* species. Phytochemistry 25, 1133–1142.

Klingauf, P., Beuerle, T., Mellenthin, A., El-Moghazy, S.A.M., Boubakir, Z., Beerhues, L., 2005. Biosynthesis of the hyperforin skeleton in *Hypericum calycinum* cell cultures. Phytochemistry (in press).

Kuhnke, J., Bohlmann, F., 1985. Synthesis of naturally occurring phloroglucinol derivatives. Tetrahedron Lett. 26, 3955–3958.

Laflamme, P., Khouri, H., Gulick, P., Ibrahim, R., 1993. Enzymatic prenylation of isoflavones in white lupin. Phytochemistry 34, 147–151.

Liu, B., Beuerle, T., Klundt, T., Beerhues, L., 2004. Biphenyl synthase from yeast-extract-treated cell cultures of *Sorbus aucuparia*. Planta 218, 492–496.

Moore, L.B., Goodwin, B., Jones, S.A., Wisely, G.B., Serabjit-Singh, C.J., Willson, T.M., Collins, J.L., Kliewer, S.A., 2000. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. PNAS 97, 7500–7502.

Müller, W.E., 2003. Current St. John's wort research from mode of action to clinical efficacy. Pharmacol. Res. 47, 101–109.

Pojer, F., Wemakor, E., Kammerer, B., Chen, H., Walsh, C.T., Li, S.M., Heide, L., 2003. CloQ, a prenyltransferase involved in clorobiocin biosynthesis. PNAS 100, 2316–2321.

Tsai, H.F., Wang, H., Gebler, J.C., Poulter, C.D., Schardl, C.L., 1995. The *Claviceps purpurea* gene encoding dimethylallyltryptophan synthase, the committed step for ergot alkaloid biosynthesis. Biochem. Biophys. Res. Commun. 216, 119–125.

Schempp, C.M., Pelz, K., Wittmer, A., Schöpf, E., Simon, J.C., 1999. Antibacterial activity of hyperforin from St. John's wort, against multiresistant *Staphylococcus aureus* and Gram-positive bacteria. Lancet 353, 2129.

Schempp, C.M., Kirkin, V., Simon-Haarhaus, B., Kersten, A., Kiss, J., Termeer, C.C., Gilb, B., Kaufmann, T., Borner, C., Sleeman, J.P., Simon, J.C., 2002. Inhibition of tumour cell growth by hyperforin, a novel anticancer drug from St. John's wort that acts by induction of apoptosis. Oncogene 21, 1242–1250.

Stanjek, V., Miksch, M., Lueer, P., Matern, U., Boland, W., 1999. Biosynthesis of psoralen: mechanism of a cytochrome P450 catalyzed oxidative bond cleavage. Angew. Chem. Int. Ed. 38, 400–402.

Wang, K., Ohnuma, S., 1999. Chain-length determination mechanism of isoprenyl diphosphate synthases and implications for molecular evolution. Trends Biochem. Sci. 24, 445–451.

Xiao, L., Tan, W., Li, Y., 1998. First total synthesis of (±)-kenusanone B. Synth. Commun. 28, 2861–2869.

Yamamoto, H., Kimata, J., Senda, M., Inoue, K., 1997. Dimethylallyl diphosphate: kaempferol 8-dimethylallyl transferase in *Epimedium diphyllum* cell suspension cultures. Phytochemistry 44, 23–28.

Yamamoto, H., Senda, M., Inoue, K., 2000. Flavanone 8-dimethylallyltransferase in *Sophora flavescens* cell suspension cultures. Phytochemistry 54, 649–655.

Yazaki, K., Kunihisa, M., Fujisaki, T., Sato, F., 2002. Geranyl diphosphate:4-hydroxybenzoate geranyltransferase from *Lithospermum erythrorhizon*. Cloning and characterization of a key enzyme in shikonin biosynthesis. J. Biol. Chem. 277, 6240–6246.

Zuurbier, K.W.M., Fung, S.Y., Scheffer, J.J.C., Verpoorte, R., 1998. In-vitro prenylation of aromatic intermediates in the biosynthesis of bitter acids in *Humulus lupulus*. Phytochemistry 49, 2315–2322.