PERGAMON

Phytochemistry 62 (2003) 121–125

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Molecules of Interest

Rosmarinic acid

Maike Petersen[a,*], Monique S.J. Simmonds[b]

[a]*Institut für Pharmazeutische Biologie, Philipps-Universität Marburg, Deutschhausstr. 17A, D-35037 Marburg, Germany*
[b]*The Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK*

Received 21 September 2002; accepted 11 October 2002

Abstract

Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid. It is commonly found in species of the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae. However, it is also found in species of other higher plant families and in some fern and hornwort species. Rosmarinic acid has a number of interesting biological activities, e.g. antiviral, antibacterial, antiinflammatory and antioxidant. The presence of rosmarinic acid in medicinal plants, herbs and spices has beneficial and health promoting effects. In plants, rosmarinic acid is supposed to act as a preformed constitutively accumulated defence compound. The biosynthesis of rosmarinic acid starts with the amino acids L-phenylalanine and L-tyrosine. All eight enzymes involved in the biosynthesis are known and characterised and cDNAs of several of the involved genes have been isolated. Plant cell cultures, e.g. from *Coleus blumei* or *Salvia officinalis*, accumulate rosmarinic acid in amounts much higher than in the plant itself (up to 36% of the cell dry weight). For this reason a biotechnological production of rosmarinic acid with plant cell cultures has been proposed.

Keywords: Antioxidant; Boraginaceae; Caffeic acid esters; Lamiaceae; Plant cell cultures; Rosmarinic acid

1. Discovery of rosmarinic acid

Even before the chemical structure of rosmarinic acid (Fig. 1) was elucidated, rosmarinic acid and similar compounds have been known as “Labiatengerbstoffe”, a type of tannin known from species of the families Lamiaceae. The tannin-like compound from *Melissa officinalis* was described to be neither a condensed catechin nor a gallo- or ellagitannin, but probably containing caffeic acid. Shortly after, two Italian chemists, Scarpati and Oriente (1958), isolated for the first time rosmarinic acid as a pure compound and named the compound rosmarinic acid according to the plant it was isolated from, *Rosmarinus officinalis*. The structure was elucidated as an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid (Scarpati and Oriente, 1958). Biogenetic studies started in 1970 when Ellis and Towers fed radioactively labelled amino acids to *Mentha* plants and showed that the two aromatic amino acids phenylalanine and tyrosine are incorporated into rosmarinic acid. Interestingly, the caffeic acid part is only formed from phenylalanine and the 3,4-dihydroxyphenyllactic acid part only from tyrosine (Ellis and Towers, 1970). These results were verified by similar feeding experiments with cell cultures of *Coleus blumei* (Razzaque and Ellis, 1977).

2. Derivatives of rosmarinic acid

A number of derivatives of rosmarinic acid (e.g. isorinic acid = 3′-deoxyrosmarinic acid, Satake et al., 1999) as well as natural products containing one or two rosmarinic acid plus other aromatic moieties have been identified from higher plants (see Fig. 1 for examples). To name all these would go beyond the scope of this article. The best known rosmarinic acid derivatives may be lithospermic acid, a conjugate of rosmarinic acid and caffeic acid, and lithospermic acid B, a dimer of rosmarinic acid (Kelley et al., 1975; Tanaka et al., 1989).

3. Distribution of rosmarinic acid in the plant kingdom

Rosmarinic acid occurs throughout the Boraginaceae, whereas within the Lamiaceae it is restricted to the subfamily Nepetoideae (Litvinenko et al., 1975). Rosmarinic

---

* Corresponding author. Tel.: +49-6421-2825821; fax: +49-6421-2825828.

E-mail address: petersen@mailer.uni-marburg.de (M. Petersen).

0031-9422/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(02)00513-7

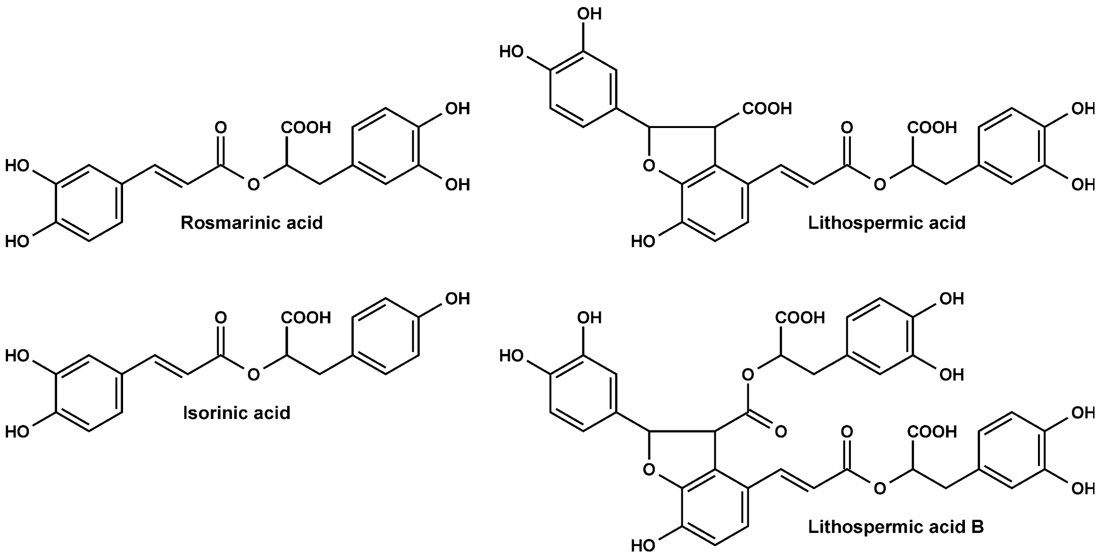

Fig. 1. Structures of rosmarinic acid and some derivatives.

acid has also been described from other plant families (Table 1). For example, it occurrs in ferns of the family Blechnaceae (Häusler et al., 1992 and literature cited therein), lower plants such as the hornworts (Takeda et al., 1990) and in monocotyledonous plants like the sea grass family Zosteraceae (Ravn et al., 1994; Petersen, unpublished results), the related Potamogetonaceae (Petersen, unpublished results) as well as the Cannaceae (Simmonds et al., submitted). This distribution shows that the occurrence of rosmarinic acid cannot be used as a chemotaxonomical marker to differentiate among families. The question remains open as to whether the ability to synthesize and/or accumulate rosmarinic acid has evolved rather early in the phylogeny of land plants or whether it has been developed independently in several taxa.

Table 1
Examples for the occurrence of rosmarinic acid in the plant kingdom

Family	Reference
Dicotyledonae	
Lamiaceae	Scarpati and Oriente, 1958; Litvinenko et al., 1975
Boraginaceae	Kelley et al., 1975
Apiaceae	Hiller, 1965
Araliaceae	Trute and Nahrstedt, 1996
Cucurbitaceae	De Tommasi et al., 1991
Hydrophyllaceae	Petersen, unpublished results
Rubiaceae	Aquino et al., 1990
Plantaginaceae	Kurkin et al., 1988 cited in Holzmannova, 1995
Sterculiaceae	Satake et al., 1999
Tiliaceae	Lasure et al., 1994
Monocotyledonae	
Cannaceae	Simmonds et al., submitted
Zosteraceae	Ravn et al., 1994
Potamogetonaceae	Petersen, unpublished results
Pteridopsida	
Blechnaceae	Häusler et al., 1992
Anthocerotopsida	
Anthocerotaceae	Takeda et al., 1990

4. Chemical synthesis and biosynthesis of rosmarinic acid

Chemical synthesis of rosmarinic acid was long sought after and was finally achieved in 1991 by Albrecht (Zinsmeister et al., 1991). Since then a number of chemical syntheses of rosmarinic acid and derivatives, e.g. the methyl ester, different stereoisomers or the less hydroxylated isorinic acid (Matsuno et al., 2002 and literature cited), have been described.

The enzymes involved in the biosynthesis of rosmarinic acid from its amino acid precursors have been unravelled in suspension cultures of *Anchusa officinalis* (Boraginaceae) and *Coleus blumei* (Lamiaceae) (see Fig. 2; Petersen et al., 1993; Petersen, 1997 and literature cited therein). Phenylalanine is transformed to an activated hydroxycinnamic acid by the enzymes of the general phenylpropanoid pathway which are already known from other biosyntheses, e.g. flavonoid or lignin biosynthesis. The first step is the deamination of L-phenylalanine to *t*-cinnamic acid by phenylalanine ammonia-lyase (PAL; E.C. 4.3.1.5). A PAL activity occurring coordinately with rosmarinic acid accumulation was for the first time shown by Razzaque and Ellis (1977). Basic characteristics of this enzyme were determined in protein preparations from a suspension cell line of *Coleus blumei* (Petersen et al., 1994). This cell line had the property to accumulate up to 21% of the dry weight as rosmarinic acid in only 10 days of cultivation when grown in a B5-based medium with high sucrose concentration (Ulbrich et al., 1985; Petersen et al., 1994). The reaction following the formation of *t*-cinnamic acid

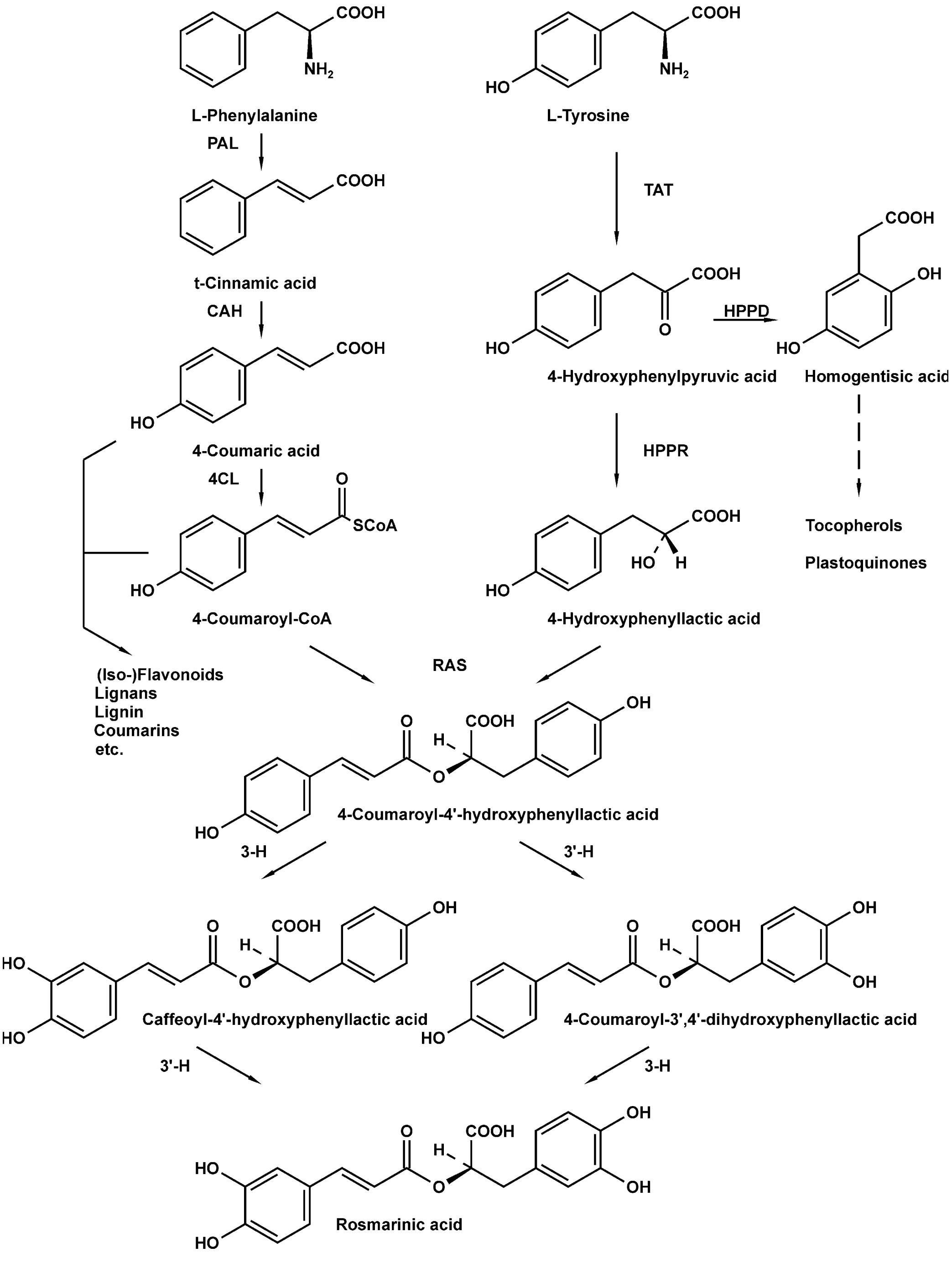

Fig. 2. Biosynthetic pathway for rosmarinic acid as found in suspension cultures of *Coleus blumei*. PAL = phenylalanine ammonia-lyase, CAH = cinnamic acid 4-hydroxylase, 4CL = hydroxycinnamate:coenzyme A ligase, TAT = tyrosine aminotransferase, HPPR = hydroxyphenylpyruvate reductase, HPPD = hydroxyphenylpyruvate dioxygenase, RAS = hydroxycinnamoyl-CoA:hydroxyphenyllactate hydroxycinnamoyl transferase, 3-H, 3′-H = hydroxycinnamoyl-hydroxyphenyllactate 3- and 3′-hydroxylases.

is its hydroxylation in position 4 to 4-coumaric acid by the cytochrome P450 monooxygenase cinnamate 4-hydroxylase (CAH; E.C. 1.14.13.11) which was also characterized from suspension cells of *Coleus blumei* (Petersen, 1997). It is generally accepted that hydroxycinnamic acids have to be activated before further reactions can take place. Several activation forms have been described. In most cases a coenzyme A thioester is formed, but also glucose esters or other cinnamic acid esters, e.g. chlorogenic acid have been shown to be able to serve as donors of hydroxycinnamic acid moieties. In *Coleus blumei*, a hydroxycinnamate:coenzyme A ligase (4CL; E.C. 6.2.1.12) involved in the biosynthesis of rosmarinic acid was characterized. The other precursor for the ester formation is formed from tyrosine. The first step is the transamination of L-tyrosine with

2-oxoglutarate as second substrate to 4-hydroxyphenylpyruvate and glutamate by the pyridoxalphosphate-dependent transaminase tyrosine aminotransferase (TAT; E.C. 2.6.1.5) (De Eknamkul and Ellis, 1987). A cDNA encoding TAT from *Coleus blumei* (EMBL GenBank Accession number AJ458993) was isolated and functionally expressed in *E. coli* recently (Lahrmann and Petersen, unpublished results). Since 4-hydroxyphenylpyruvate is not an intermediate of aromatic amino acid biosynthesis in plants, it has to be formed from tyrosine as described above. In addition to rosmarinic acid biosynthesis it serves as precursor for homogentisic acid, a precursor for the essential metabolites tocopherols and plastoquinones. The cDNA encoding hydroxyphenylpyruvate dioxygenase (HPPD; E.C. 1.13.11.27) involved in the formation of homogentisic acid has been isolated from *Coleus blumei* (EMBL GenBank Accession number AJ309203; Kim, 2002). For rosmarinic acid biosynthesis, 4-hydroxyphenylpyruvate is reduced to the corresponding 4-hydroxyphenyllactate by hydroxyphenylpyruvate reductase (HPPR; E.C. 1.1.1.237; Petersen et al., 1993). The enzyme accepts NADH as well as NADPH as co-substrates and also reduces 3,4-dihydroxyphenylpyruvate although with a lower affinity. A full-length cDNA for HPPR was recently isolated from *Coleus blumei* (EMBL GenBank Accession number AJ507733) and functionally expressed in *E. coli* (Kim, 2002). The resulting hydroxyphenyllactate is the *R*(+)-stereoisomer, and this is the only isomer accepted by the ester-forming enzyme hydroxycinnamoyl-CoA:hydroxyphenyllactate hydroxycinnamoyl transferase (trivial name: rosmarinic acid synthase (RAS; E.C. 2.3.1.140); Petersen et al., 1993). Under release of coenzyme A the ester linkage is formed between the carboxyl group of 4-coumaric acid and the aliphatic hydroxyl group of 4-hydroxyphenyllactate. The resulting ester is 4-coumaroyl-4′-hydroxyphenyllactate which is afterwards hydroxylated in positions 3 and 3′ of the aromatic rings by two cytochrome P450 monooxygenases (Petersen, 1997). A cDNA for a cytochrome P450 catalyzing the 3-hydroxylation of the 4-coumaric acid part of the ester to a caffeic acid part was recently isolated from *Lithospermum erythrorhizon* (CYP98A6; Matsuno et al., 2002). A number of cDNAs encoding cytochrome P450s that are able to hydroxylate 4-coumaric acid or a 4-coumaroyl-moiety in an ester (e.g. 4-coumaroyl-shikimate) have been published recently (Schoch et al., 2001; Franke et al., 2002; Anterola et al., 2002). These cytochrome P450s are all grouped into the CYP98 family. A CYP98A14 with certain homology to the published sequences (see above) has been isolated from rosmarinic acid-synthesising suspension cells of *Coleus blumei* (EMBL accession number AJ427452; Petersen, unpublished result).

5. Biotechnological production of rosmarinic acid by plant in vitro cultures

Rosmarinic acid is readily accumulated in undifferentiated plant cell cultures, in some cases in concentrations much higher than in the plant itself. The first plant cell cultures accumulating rosmarinic acid were derived from *Coleus blumei* (Razzaque and Ellis, 1977; Zenk et al., 1977). Suspension cultures from this species were the first ones used for an attempted biotechnological production of rosmarinic acid since they were found to accumulate up to 21% rosmarinic acid in the dry weight. Ulbrich et al. (1985) reported on the isolation of about 100 g crystalline rosmarinic acid from suspension cells cultivated in a two-stage process in bioreactors of 32 l working volume for 14 days. Cell cultures of other species of plants were then used for rosmarinic acid production. The highest content of rosmarinic acid at 36% of the cell dry weight was described by Hippolyte et al. (1992) in a suspension culture of *Salvia officinalis* in a low osmolarity medium with 5% sucrose.

The effect of increased sucrose concentrations on rosmarinic acid accumulation was elucidated by Gertlowski and Peterson (1993) in a suspension culture of *Coleus blumei*. The level of rosmarinic acid accumulation was shown to be dependent on the sucrose content, whereas the growth limitation (e.g. by a limiting supply of phosphate) determined the onset of rosmarinic acid accumulation. Several authors showed that rosmarinic acid accumulation can furthermore be enhanced by the addition of fungal elicitors (e.g. yeast extract, preparations from *Pythium aphanidermatum*) or methyl jasmonate (see Szabo et al., 1999 and literature cited therein). This suggests that rosmarinic acid could have a role in plants as a defence compound against pathogens and herbivores. In fact, recent research has shown that rosmarinic acid is a potent phagodeterrent against the tobacco hornworm, *Manduca sexta* (Simmonds et al., submitted). It deters these larvae from feeding but if eaten is not toxic. We could find no other information in the literature about the effect of rosmarinic acid on other herbivores.

6. Biological activity of rosmarinic acid

A multitude of biological activities have been described for rosmarinic acid and a comprehensive description would go beyond the scope of this article. Main activities are adstringent, antioxidative, antiinflammatory, antimutagen, antibacterial and antiviral (e.g. Parnham and Kesselring, 1985). The latter activity is used in the therapy of *Herpes simplex* infections with rosmarinic acid-containing extracts of *Melissa officinalis*. The antiinflammatory properties are thought to be based on the inhibition of lipoxygenases and cyclooxygenases and the interference of rosmarinic acid with the complement cascade (Parnham and Kesselring, 1985). Rosmarinic acid is rapidly eliminated from the blood circulation after intravenous administration ($t\ \frac{1}{2} = 9$ min) and shows a very low toxicity with a LD_{50} in mice of 561 mg kg^{-1} after intravenous application (Parnham and Kesselring, 1985). Phenolic compounds like rosmarinic acid can provide protection against cancer and rosmarinic acid contributes to the anti-oxidant activity of plants used in the cosmetic industry, such as *Rosmarinus officinalis* and *Sanicula europaea* (D'Amelio, 1999).

References

Anterola, A.M., Jeon, J.H., Davin, L.B., Lewis, N.G., 2002. Transcriptional control of monolignol biosynthesis in *Pinus taeda*. Factors affecting monolignol ratios and carbon allocation in phenylpropanoid metabolism. J. Biol. Chem. 277, 18272–18280.

Aquino, R., Ciavatta, M.L., De Simone, F., Pizza, C, 1990. A flavanone glycoside from *Hamelia patens*. Phytochemistry 29, 2358–2360.

D'Amelio, F.S., 1999. Botanicals, A Phytocosmetic Desk Reference. CRC Press, London, pp. 361.

De Eknamkul, W., Ellis, B.E., 1987. Tyrosine aminotransferase: the entrypoint enzyme of the tyrosine-derived pathway in rosmarinic acid biosynthesis. Phytochemistry 26, 1941–1946.

De Tommasi, N., De Simone, F., De Feo, V., Pizza, C., 1991. Phenylpropanoid Glycosides and Rosmarinic Acid from *Momordica balsamina*. Planta Med. 57, 201.

Ellis, B.E., Towers, G.H.N., 1970. Biogenesis of rosmarinic acid in *Mentha*. Biochem. J. 118, 291–297.

Franke, R., Humphreys, J.M., Hemm, M.R., Denault, J.W., Ruegger, M.O., Cusumano, J.C., Chapple, C., 2002. The Arabidopsis REF8 gene encodes the 3-hydroxylase of phenylpropanoid metabolism. Plant J. 30, 33–45.

Gertlowski, C., Petersen, M., 1993. Influence of the carbon source on growth and rosmarinic acid production in suspension cultures of *Coleus blumei*. Plant Cell Tiss. Org. Cult. 34, 183–190.

Häusler, E., Petersen, M., Alfermann, A.W., 1992. Rosmarinsäure in *Blechnum*-Spezies. In: Haschke, H.P., Schnarrenberger, C. (Eds.), Botanikertagung. Berlin. Akademie Verlag, Berlin, p. 507.

Hiller, K., 1965. Zur Kenntnis der Inhaltsstoffe einiger Saniculoidae. 1. Mitteilung: *Sanicula europaea* L.—Isolierung und quantitative Erfassung von Chlorogen-und Rosmarinsäure. Pharmazie 20, 574–579.

Hippolyte, I., Marin, B., Baccou, J.C., Jonard, R., 1992. Growth and rosmarinic acid production in cell suspension cultures of *Salvia officinalis* L. Plant Cell Rep. 11, 109–112.

Holzmannova, V., 1995. Kyselina rosmarinová její biologická aktivita. Chem. Listy 90, 486–496.

Kelley, C.J., Mahajan, J.R., Brooks, L.C., Neubert, L.A., Breneman, W.R., Carmack, M., 1975. Polyphenolic acids of *Lithospermum ruderale* Dougl. ex Lehm. (Boraginaceae). 1. Isolation and structure determination of lithospermic acid. J. Org. Chem. 40, 1804–1815.

Kim, K.H., 2002. Hydroxyphenylpyruvate Reductase (HPPR) and Hydroxyphenylpyruvate Dioxygenase (HPPD) from Suspension Cultures of *Coleus blumei*: Purification–Cloning–Expression. PhD thesis, University of Marburg, Germany.

Lasure, A., Van Poel, B., Pieters, L., Claeys, M., Gupta, M., Vanden Berghe, D., Vlietinck, A.J., 1994. Complement-inhibiting properties of *Apeiba tibourbou*. Planta Med. 60, 276–277.

Litvinenko, V.I., Popova, T.P., Simonjan, A.V., Zoz, I.G., Sokolov, V.S., 1975. "Gerbstoffe" und Oxyzimtsäureabkömmlinge in Labiaten. Planta Med. 27, 372–380.

Matsuno, M., Nagatsu, A., Ogihara, Y., Ellis, B.E., Mizukami, H., 2002. *CYP98A6* from *Lithospermum erythrorhizon* encodes 4-coumaroyl-4′-hydroxyphenyllactic acid 3-hydroxylase involved in rosmarinic acid biosynthesis. FEBS Lett. 514, 219–224.

Parnham, M.J., Kesselring, K., 1985. Rosmarinic acid. Drugs of the Future 10, 756–757.

Petersen, M., 1997. Cytochrome P-450-Dependent hydroxylation in the biosynthesis of rosmarinic acid in *Coleus*. Phytochemistry 45, 1165–1172.

Petersen, M., Häusler, E., Karwatzki, B., Meinhard, J., 1993. Proposed biosynthetic pathway for rosmarinic acid in cell cultures of *Coleus blumei* Benth. Planta 189, 10–14.

Petersen, M., Häusler, E., Meinhard, J., Karwatzki, B., Gertlowski, C., 1994. The biosynthesis of rosmarinic acid in suspension cultures of *Coleus blumei*. Plant Cell Tiss. Org. Cult. 38, 171–179.

Ravn, H., Pedersen, M.F., Andary, J., Borum, C., Anthoni, U., Christophersen, C., Nielsen, P.H., 1994. Seasonal variation and distribution of two phenolic compounds, rosmarinic acid and caffeic acid, in leaves and roots-rhizomes of eelgrass (*Zostera marina* L.). Ophelia 40, 51–61.

Razzaque, A., Ellis, B.E., 1997. Rosmarinic acid production in *Coleus* cell cultures. Planta 137, 287–291.

Satake, T., Kamiya, K., Saiki, Y., Hama, T., Fujimoto, Y., Kitanaka, S., Kimura, Y., Uzawa, J., Endang, H., Umar, M., 1999. Studies on the constituents of fruits of *Helicteres isora* L. Chem. Pharm. Bull. 47, 1444–1447.

Scarpati, M.L., Oriente, G., 1958. Isolamento e costituzione dell'acido rosmarinico (dal *rosmarinus off.*). Ric. Sci. 28, 2329–2333.

Schoch, G., Goepfert, S., Morant, M., Hehn, A., Meyer, D., Ullmann, P., Werck-Reichhart, D., 2001. CYP98A3 from *Arabidopsis thaliana* is a 3′-hydroxylase of phenolic esters, a missing link in the phenylpropanoid pathway. J. Biol. Chem. 276, 36566–36574.

Simmonds, M.S.J., Stevenson, P.C., Hanson, F.E. Rosmarinic acid in *Canna generalis* (Cannaceae) activates the medial deterrent chemosensory neurone and deters feeding in the tobacco hornworm, *Manduca sexta* (Lepidoptera). Physiological Entomology (submitted).

Szabo, E., Thelen, A., Petersen, M., 1999. Fungal elicitor preparations and methyl jasmonate enhance rosmarinic acid accumulation in suspension cultures of *Coleus blumei*. Plant Cell Rep. 18, 485–489.

Takeda, R., Hasegawa, J., Sinozaki, M., 1990. The first isolation of lignans, megacerotonic acid and anthocerotonic acid, from non-vascular plants, Anthocerotae (hornworts). Tetrahedron Lett. 31, 4159–4162.

Tanaka, T., Morimoto, S., Nonaka, G., Nishioka, I., Yokozawa, T., Chung, H.Y., Oura, H., 1989. Magnesium and ammonium-potassium lithospermates B, the active principles having a uremia-preventive effect from *Salvia miltiorrhiza*. Chem. Pharm. Bull. 37, 340–344.

Trute, A., Nahrstedt, A., 1986. Separation of rosmarinic acid enantiomers by three different chromatographic methods (HPLC, CE, GC) and the determination of rosmarinic acid in *Hedera helix* L. Phytochem. Anal. 7, 204–208.

Ulbrich, B., Wiesner, W., Arens, H., 1985. Large scale production of rosmarinic acid from plant cell cultures of *Coleus blumei* Benth. In: Neumann, K.H., Barz, W., Reinhard, E. (Eds.), Primary and Secondary Metabolism of Plant Cell Cultures. Springer, Berlin, Heidelberg, pp. 293–303.

Zenk, M.H., El-Shagi, H., Ulbrich, B., 1977. Production of rosmarinic acid by cell-suspension cultures of *Coleus blumei*. Naturwissenschaften 64, 585–586.

Zinsmeister, H.D., Becker, H., Eicher, T., 1991. Moose, eine Quelle biologisch aktiver Naturstoffe? Angew. Chem. 103, 134–151.