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High Purity *cis*-Cinnamic Acid Preparation for Studying Physiological Role of *trans*-Cinnamic and *cis*-Cinnamic Acids in Higher Plants

María Laura SALUM and Rosa ERRA-BALSELLS

CIHIDECAR-CONICET, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón II, 3 P, Ciudad Universitaria, 1428-Buenos Aires, Argentina

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Cinnamic acids are present in all kinds of plant tissues and as consequence in herbs and derived medicines, cosmetics, foods, etc. The interest in study their role in nature and possible health effect on consumers has grown exponentially. Because of their molecular structure they can exist in both *trans*- and *cis*-forms, having both found in plants. However, as only the *trans*-forms are commercially available very few studies *in vitro* and *in vivo* of the *cis*-role are available. In the present review the current knowledge in this field of studies is summarized including the brief description of a new tool for easy and friendly synthesis of *cis*-cinnamic acids by researchers in order to be able to conduct new experiments.

Keywords : caffeic acid, coumaric acid, ferulic acid, geometric isomers, sinapinic acid

INTRODUCTION

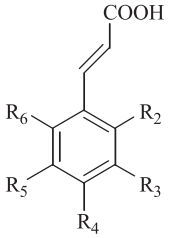
Plants originated in aquatic environment successfully achieved adaptation to land because of massive biosynthesis of phenolic compounds (Buchanan et al., 2000). Although majority of these substances assumed cell wall structural roles, a vast array of nonstructural constituents was also formed, having such various roles as defending plants, determining certain distinguish features of different woods and barks (i.e., durability), establishing flower color and contributing to certain flavors (taste and odors), etc. These functions and other performed by plant phenolic compounds are essential for the continued survival of all types of vascular plants. Plant phenolics are generally characterized as aromatic metabolites that possess, or formerly possessed, one or more acidic hydroxyl groups attached to the aromatic arene (phenyl) ring (see Table 1). These compounds plagued plant scientists for a long time by interfering with experimental methods: i.e., when exposed to air, plant phenolics readily oxidize and turn brown generating products that form complexes with proteins and inhibit enzyme activity; cultured plant tissues can also release phenolic compounds that inhibit growth of callus and regeneration of plantlets (Buchanan et al., 2000). Simultaneously, phenolic compounds are increasingly being recognized for their profound impact on plant growth, development, reproduction, and defense. Indeed, scientists have come to appreciate their significance more fully, particularly over the past few decades. The discussion of plant phenolics substances is a discussion of plant diversity itself. Characteristics unique to each of roughly 250,000 species of vascular plants arise, at least in part, through differential deposition of highly specialized hydroxyphenyl and phenylpropanoid derivatives. In ferns, fern allies, and seed plants, polymeric lignins reinforce specialized cell walls, enabling them to support their massive weights on land and to transport water and minerals from roots to leaves. Closely realted to lignins, the lignans can vary from dimers to high oligomers. Widespread throughout the plant kingdoms, lignans for example can either help defend against various pathogens or act as antioxidants in flowers, seeds, seed coats, stems, nuts, barks, leaves and roots. Suberized tissues contain layers of hydrophobic (aliphatic) and hydrophilic (phenolic) structural substances. Present in cork, bark, roots, and certain periderm tissues (i.e., potato skin), these tissues function, for example provide a protective barrier limiting the effects of desiccation and pathogen attack. Among the phenolic compounds, flavonoids comprise an astonishing family of more than 4500 compounds with quite different roles (pigments, feeding deterrents and wood protectants, defensive, signaling molecules, etc.) (Buchanan et al., 2000; Taiz and Zeiger, 2006).

Plant phenolic compounds are biosynthetized by several different routes. Two basic pathways are involved: the shikimic acid pathway and the malonic acid pathway. The former participates in the biosynthesis of most plant phenolics. The latter is important in fungi and bacteria but of less significance in higher plants. The shikimic acid pathway converts simple carbohydrate precursors derived from glycolysis and the pentose phosphate pathway to the aromatic amino acids. This pathway is present in plants, fungi and bacteria (Buchanan et al., 2000; Taiz and Zeiger, 2006).

Although most plant phenolic compounds are products of phenylpropanoid metabolism, with the phenylpropanoids, in turn, being derived from phenylalanine and ty-

Corresponding author : R. Erra-Balsells, fax: +54–11–45763346,
e-mail : erra@qo.fcen.uba.ar

Table 1 Chemical structure of some *trans*-cinnamic acids (*trans*-CH) present in plants.



trans-CH

	Acid	Abbreviation	R^2	R^3	R^4	R^5	R^6
1	Cinnamic	CA	H	H	H	H	H
2	Coumaric	CuA	H	H	OH	H	H
3	Ferulic	FA	H	OCH_3	OH	H	H
4	5-Hydroxy-ferulic	5HFA	H	OCH_3	OH	OH	H
5	Sinapinic	SA	H	OCH_3	OH	OCH_3	H
6	3,4-Dioxymethylen-cinanmic	3,4MDCA	H	$-CH_2$	OCH_{2-}	H	H
7	Caffeic	CfA	H	OH	OH	H	H
8	3-Hyidroxy-4-methoxy-cinnamic	iFA	H	OH	OCH_3	H	H
9	3-Hydroxy-cinnamic	3HCA	H	OH	H	H	H
10	2-Hydroxy-cinnamic	2HCA	OH	H	H	H	H

rosine, some phenolic compounds are generated by alternative pathways; i.e., hydrolizable tannins, typically copolymers of carbohydrates and gallic and ellagic acids (shikimate derivatives) found in leaves, fruits, pods and galls of some woody dicots, have not been yet found in monocots (Buchanan et al., 2000). On the other hand condensed tannins are widespread and are present in all trees and shrubs (Buchanan et al., 2000; Taiz and Zeiger, 2006).

One enzyme directs carbon from aromatic amino acids to the synthesis of phenylpropanoid metabolites. This enzyme called phenylalanine ammonia lyase (PAL) converts phenylalanine (Phe) to cinnamic acid (CA) and the analogue enzyme called tyrosine ammonia lyase (TAL), tyrosine (Tyr) to p-coumaric acid (p-CuA) (see Table 1, and figures and discussion below). Interestingly, in most vascular plants, Phe is the highly preferred substrate, but the monocot enzyme can utilize both Phe and Tyr. PAL has been detected in a few aquatic plants, where it probably functions in formation of simple flavonoids. Thus, this PAL (TAL) enzymatic step and the products of the various phenylpropanoid and phenylpropanoid-acetate pathways appear to have been a key to plant colonization of land (Buchanan et al., 2000; Taiz and Zeiger, 2006).

Impressive progress was made in defining salient features of the pathway that converts cinnamic acid to cinnamic acid derivatives which then will be components of the lignols structure. This pathway comprises four types of enzymatic reactions: aromatic hydroxylation, O-methylation, CoA ligations and NADPH-dependent reductions. Aromatic ring hydroxylation involves three distinct hydroxylation conversions, all of which are believed to be microsomal (Buchanan et al., 2000).

Cinnamic acids (*CHs*)

Phenylpropanoid acids called cinnamic acids (CHs) are present in all kind of plant cells and tissues. Because of their 1,2-disubstituted alkenic molecular structure they

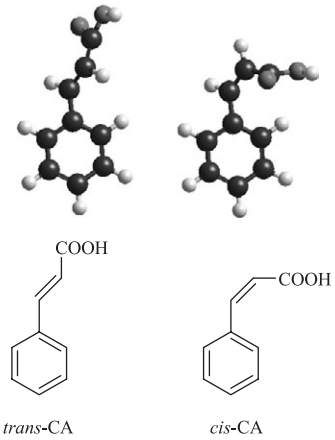


Fig. 1 Structure of *cis*-cinnamic (*cis*-CA) and *trans*-cinnamic (*trans*-CA) acids. Tridimensional (upper) and flat (botton) display.

can exist in both geometric molecular arrangements (geometric isomers): *trans*- and *cis*-forms (Taiz and Zeiger, 2006), both forms have been found in nature. However, at the moment of isolation and characterization, the *trans*-cinnamic acids have been shown to be the predominant form in the extracts, may be because they are much more stable than the corresponding *cis*-isomer or because of their biological role (Sun et al., 2002). In Fig. 1 the molecule structure of the most simple phenylpropanoid acid, the cinnamic acid (CA), is shown.

Cinnamic acids are secondary metabolites produced by plants. They are involved in the pathway responsible for the biosynthesis of numerous biomolecules such phenolic compounds, flavonoids, stilbene, salisylic acid and lignin (Buchanan et al., 2000; Taiz and Zeiger, 2006). This compounds play important roles in plant growth and in plant-environment interactions and also functions as cell wall components, anti-herbivore compounds, UV protectants,

antioxidants, pigments, aroma compounds and so on (Buchanan et al., 2000; Dixon, 2001; Taiz and Zeiger, 2006). Most of these products biosynthesized from the phenylpropanoic pathway contain carbon-to-carbon double bonds (1,2-disubstituted alkene moiety). Thus *cis/trans*-isomers of this class of compounds, such as cinnamic acid (CA), coumaric acid (CuA), caffeic acid (CfA) and ferulic (FA) acid (Table 1), have been detected from a large number of dicots and monocots (Guo et al., 2011).

Cinnamic acids role in plant physiology

The key intermediate of phenyl propanoid pathway, *trans*-cinnamic acid (Table 1, *trans*-CA), is synthesized from L-phenylalanine (L-Phe), which is catalyzed by PAL and converted to *trans*-4-hydroxycinnamic acid (*trans*-coumaric acid, *trans*-CuA) by CA 4-hydroxylase (C4H) (Fig. 2) (Buchanan et al., 2000; Taiz and Zeiger, 2006).

The *trans*-CuA is also synthesized from tyrosine (Tyr) catalyzed by tyrosine ammonia lyases (TAL) (Fig. 3) (Graft, 1992).

trans-4-Hydroxy-3-methoxycinnamic acid (*trans*-ferulic acid, *trans*-FA) derives from *trans*-CuA through enzymatic hydroxylation followed by methylation with methionine acting as the methyl donor species, catalyzed by s-adenosyl-L-methionine: *trans*-3,4-dihydroxy-cinnamic acid (*trans*-caffeic acid, *trans*-CfA) 3-o-methyl-transferase (COMT) (Fig. 4) (Buchanan et al., 2000).

This bifunctional enzyme also produces the methylation of *trans*-5-hydroxy-ferulic acid (*trans*-5HFA) to give *trans*-4-hydroxy-3,5-dimethoxycinnamic acid (*trans*-sinapinic acids, *trans*-SA) (Fig. 5) (Raes et al., 2003).

Over the last decade, there has been a tremendous effort in cloning new genes involved in the monolignol biosynthetic pathway and in tackling the enzyme kinetics of the corresponding proteins and the role that the enzymes play in controlling the amount and composition of lignin to be deposited in the cell wall. As a consequence, the monolignol biosynthetic pathway has virtually been rewritten, although the exact route toward the monolignols is still a matter of debate (Fig. 6) (Raes et al., 2003).

It is important to note that only *trans*-cinnamic-like structure species are involved in this biosynthetic net (biosynthesis pathways).

However, the *cis*-form of CA, identified from oil of *Alpinia malacensis*, could be a product of photoisomerization of *trans*-CA as it was produced *in vitro* (Yang et al., 1999). As *cis*-CA was believed to be extremely scarce in nature and trace amount of free considered to be insufficient to have any physiological implication, this unique compounds has therefore been perceived as a synthetic plant growth regulation for decades (Yin et al., 2003). For this very reason, little effort has been devoted to study the production and function of this plant growth regulator in higher plants in the past years and few researches have been conducted to show the physiological roles of *cis*-CA in plants (Yin et al., 2003). Yin et al. report an unexpected finding that *Brassica parachinensis* plant contains the naturally occurring *cis*-CA (Yin et al., 2003). The concentration of *cis*-CA in *Brassica* plant appears to be comparable to physiologically effective concentrations of major plant hormones (Yin et al., 2003). These authors propose four possible pathways for production of *cis*-CA, as follows: i) a sunlight-mediated conversion from *trans*-CA; ii) a spontaneous conversion from *trans*-CA in the presence of electron-tranfer sensitizer and/or photosensitizers; iii) a product of isomerase catalysis from *trans*-CA, and iv) a product of enzyme catalysis from L-phenylalanine (Fig. 7). Similarly, *cis*-form of other phenylpropanoids, such as cis-CuA, *cis*-FA and *cis*-caffeic acid, have also been identified from plants (Wong et al., 2005).

Nowadays, it is known that biological properties of *cis*-CA are distinctly different from those of *trans*-CA, and that it plays a differential role in regulations of plant

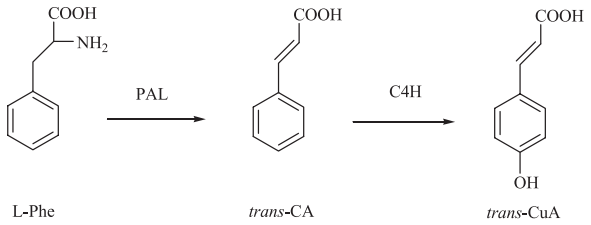


Fig. 2 *trans*-Coumaric acid (*trans*-CuA) from *trans*-cinnamic acid (*trans*-CA). (Young and Neish, 1966; Buchanan et al., 2000; Taiz and Zeiger, 2006) .

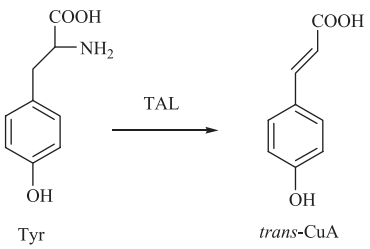


Fig. 3 *trans*-Coumaric acid from Tyrosine (Tyr). (Graft, 1992).

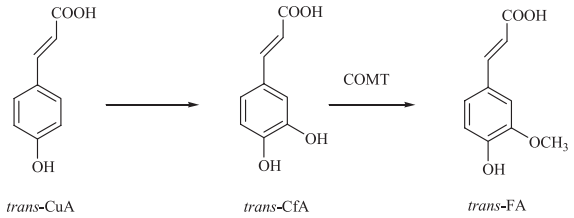


Fig. 4 *trans*-Ferulic acid (*trans*-FA) from *trans*-coumaric acid (*trans*-CuA). (Buchanan et al., 2000; Taiz and Zeiger, 2006).

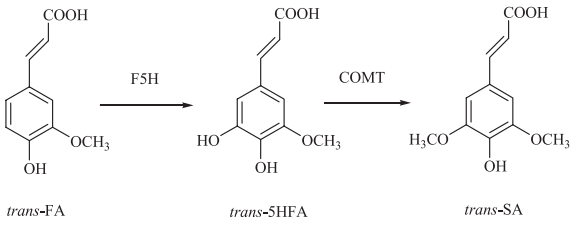


Fig. 5 *trans*-Sinapinic acid (*trans*-SA) from *trans*-ferulic acid (*trans*-FA) through *trans*-5HFA (trans-5HFA) (F5H; COMT). (Buchanan et al., 2000; Raes et al., 2003; Taiz and Zeiger, 2006).

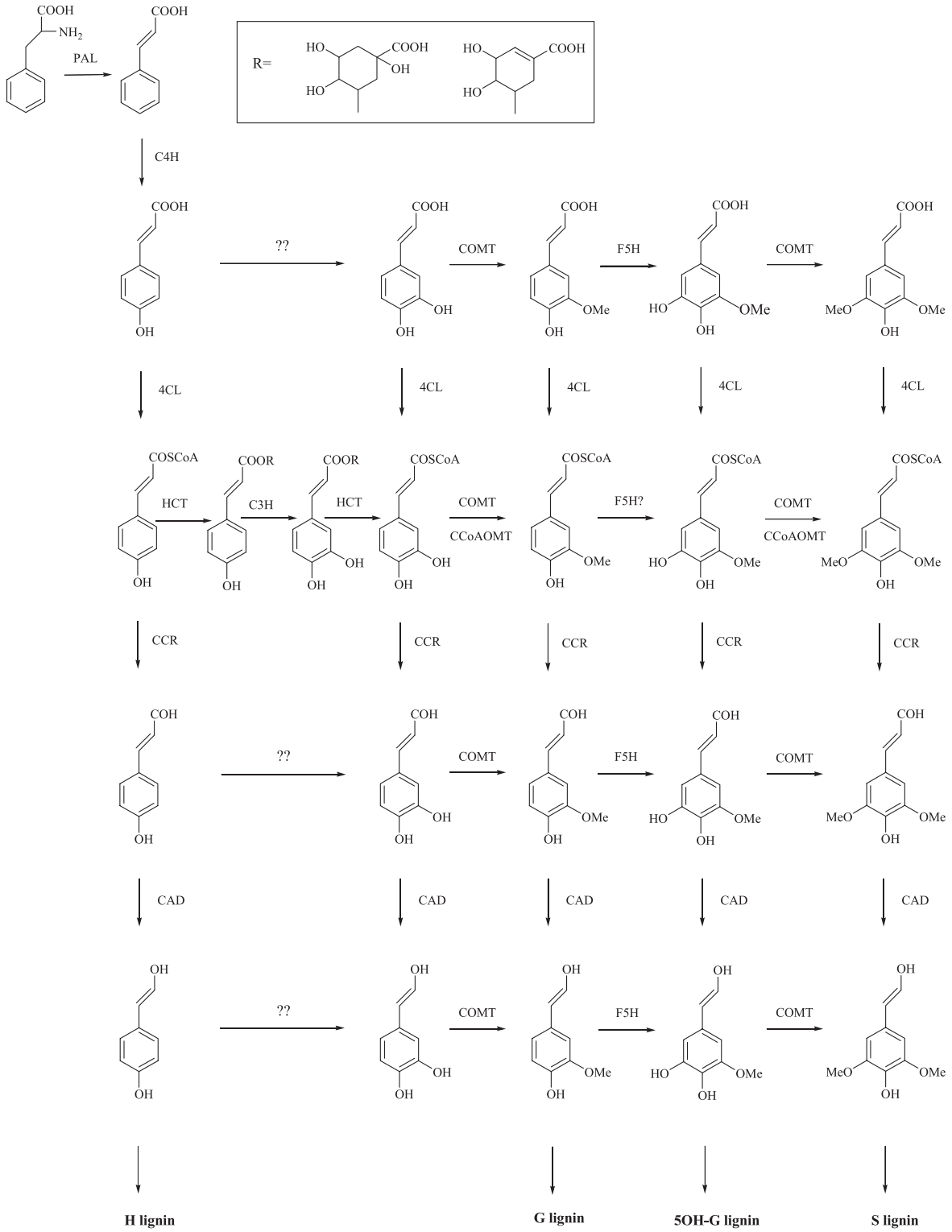


Fig. 6 The monolignol biosynthetic pathway. All the enzymatic reactions presented in the pathway have been demonstrated at least *in vitro* (PAL, phenylalanine ammonia lyase; C4H: cinnamae-4-hydroxylase (O_2, cytochrome P450, NADPH); COMT: O methyltransferases; F5H: hydroxylases (O_2, cyt. P450, NADPH); 4CL: CoA ligases for AMP and CoA (CoASH, ATP) ligation; HCT and C3H: CoA ligases for ligation of AMP and CoA (CoASH, ATP); CCR: cinnamoy-CoA:NADPH oxidareductases; CAD: cinnamoyl alcohol dehydrogenases (NADPH)). (Buchanan et al., 2000; Raes et al., 2003; Taiz and Zeiger, 2006).

growth. *trans*-CA has been found to inhibit the growth of rice (*Oryza sativa* L.) seedling, promote root formation, stimulate seed germination, induce sporulation of *Pyricularia spp*. and inhibit germination of *Collectotrichum falcatum* spores (Letham, 1978). *trans*-CA is generally considered to be either inactive as an auxin-like compound or antagonistic to the effects of auxin (Åberg, 1961; Letham, 1978; Yang et al., 1999). In contrast, it is known that synthetic *cis*-CA has a growth promoting activity in plants such as avena (*Avena sativa*) (Haagen-Smit and

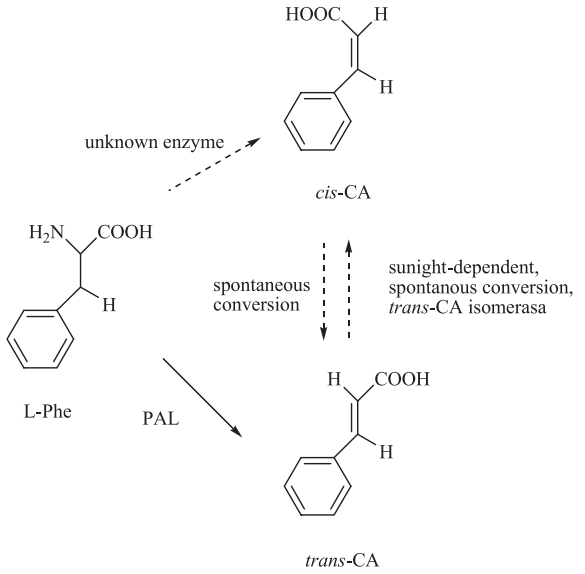


Fig. 7 Possible pathways for production of *cis*-cinnamic acid (*cis*-CA). (Buchanan et al., 2000; Yin et al., 2003; Taiz and Zeiger, 2006).

Went, 1935; Åberg, 1961). The *cis*-CA was also shown to inhibit root growth of wheat (*Tritiucum aestivium* L.) (Åberg, 1961), and flax (*Linum usitatissimum*) (Åberg, 1961). Furthermore, the vapor of cis-CA inhibited the negative gravitropic response of stems of *Arabidopsis thaliana* (Yang et al., 1999) and tomato (*Lycopersicon esculentum* Mill.) (Yang et al., 1999) and delays ethylene surge during banana fruit (got from local market) ripening (Yang et al., 1999). These results support the conclusion that the action sites of the vapor of *cis*-CA and ethylene are fundamentally different (Yang et al., 1999). Further study has shown that *cis*-CA acts on plant cell through both ethylene- and auxin-independent signaling pathways (Wong et al., 2005). These biological proprieties of *cis*-CA are distinctly different from those of *trans*-CA in plants, which is generally believed to be physiologically inactive and be antagonistic function to the effect of auxin in higher plants (Wong et al., 2005). These results suggest that *cis*-CA is a member of the super group of auxin-like substances including indole acids, naphthalene acids, chlorophenoxy acids, benzoic acids, picolinic acid derivatives, amino acids and amines. It possesses the unique structural characteristics of these auxin-like substances, which were described by Veldstra (Veldstra, 1944) as a basal ring system (non-polar part) with high interface activity and a carboxyl group (polar part), generally an acidic moiety, oriented in particular angle with respect to the plane of ring system (Yang et al., 1999). Thus, *cis*-CA is auxin-like but lacks a polar transport property. To elucidate the mode of action of *cis*-CA, Yang et al. (1999) proposed four possible action pathways: i) *cis*-CA might act through the induction of ethylene biosynthesis, which trigger the observed plant responses; ii) It might act as an ethylene analog and directly interact with ethylene receptor; iii) It might interact with the downstream components of ethylene signaling pathway; iv) It might act through an ethylene-independent pathway, for example, an auxin-dependent pathway (Yang et al., 1999). Treatment of *Arabidopsis thaliana* L. *Heynh* (Coumbia-0 ecotype) seedling with ultraviolet (UV)-light in the presence of pieronylic acid (PA), which inhibits C4H activity and results in an *in plant* accumulation of *trans*-CA, is able to increase the ratio of *cis*-CA over *trans*-CA (Wong et al., 2005). Combination of UV irradiation with PA treatment inhibits the root growth of *Arabidopsis* seedlings. Similarly, *cis*-CA was also detected from rice (*Oryza sativa* L.) seedlings grown under sunlight (Wong et al., 2005). These results suggest that *cis*-CA may be produced and function in plants and it may play a role in plant growth. To seek molecular evidences for the action of this phenylpropanoic compound from *Arabidopsis*, the light-grown seedlings were treated with mixture of CA acids, because naturally occurring *cis*-CA is in accompany with *trans*-CA in plants (Yin et al., 2003; Wong et al., 2005), demonstrating that *cis*-CA also has strong inhibitory effect on the aerial part of the plants (Guo et al., 2011). However, little is known about the genes that are regulated either by *cis*-CA or *cis*-phenylpropanoids in general.

cis- and *trans*-CA have different effects on the catalytic activity of PAL, the enzyme responsible for synthesis of CA from Phe. Chen et al. (Chen et al., 2005) investigated the effect of *cis*-CA on the activity of *Arabidopsis* PAL gene family (*AtPAL1*, *AtPAL2*, *AtPAL4*). These results showed that *cis*-CA is a competitive inhibitor for PAL 1, but not PAL 2 and PAL 4, whereas *trans*-CA acts as a competitive inhibitor for all three PAL isomers (Chen et al., 2005). Although *trans*-CA regulates PAL activity through negative feedback, the *cis*-isomer had a much less inhibitory effect on PAL enzyme activity; it is therefore possible that conversion of *trans*-CA to *cis*-CA in plant cells may function as one of the mechanisms that rapidly ameliorate the negative feedback effect of *trans*-CA on PAL activity at the enzyme level (Chen et al., 2005). However, in the recent studies, both *cis*-CA and *trans*-CA long-term did not exert a negative feedback effect on PAL1 and PAL 2 transcript level (Guo et al., 2011). Guo et al. (2011) report the proteomics-based identification of two *cis*-CA enhancement genes (Zusammen-CA-Enhanced, *ZCE*), from a model *Arabidopsis* and provide the *in vivo* evidence for a role for these genes in regulation of bolting. The 1,241 *Arabidopsis* seedling proteins identified via proteomic, 1,196 (96%) of them are considered to be irrelevant to *cis*-CA regulation (Guo et al., 2011). Bionformatic analysis of *ZCE1* and *ZCE2* gene reveals that the two genes belong to the super gene family of the major latex protein (*MLP*)-like proteins, consisting of 23 members in *Arabidopsis*. Phylogenetic analysis of these *MLP-Like (MLPL)* protein members reveal that *ZCE1 (MLPL1)* and *ZCE2 (MLPL2)* are closely related to each other in the primary sequence (Guo et al., 2011). These results may provide some insight into the molecular mechanism by which *cis*-CA or its phenylpropanoid derivatives (i.e., *cis*-CuA, *cis*-FA, *cis*-CFA, *cis*-SA and so on), regulate plant growth and development as well as plant adaptation to environmental stresses (Guo et al., 2011). *cis*-CA more enhancer the transcript level of *ZCE1* and *ZCE2* than *trans*-CA, the mixed CA isomers, in an equal amount, enhanced also the

transcript level of this genes (Guo et al., 2011). The effects of *cis/trans*-CA as well as those of some of their phenylpropanoid derivatives, *trans*-CuA, *trans*-FA and *trans*-SA and their counterpart *cis*-isoform were also comparative studied on gene expression (Guo et al., 2011). Treatment of *Arabidopsis* seedlings with these *cis/trans*-phenylpropanoids revealed that most of them are to enhance ZCE1 transcript level. The highest induction was achieved again by treatment with *cis*-CA, followed by the *cis*-SA and to lesser extent by *cis*-FA acid and *cis*-CuA acid; the authors description and results are summarized and very clearly displayed in figures (Guo et al., 2011). Since the purity of cinnamic acids used in the experiment was not 100%, it is therefore concluded that other *cis*-phenylpropanoids do not possess such strong effect on enhancement of ZCE1 and ZCE2 transcripts as *cis*-CA does. Indeed, it was reported that hydroxylation on ring reduces the activity of cinnamic acid (Guo et al., 2011).

It has been reported that *cis*-CA forms conjugates with glucose to become *cis*-cinnamoyl glucosides, which has been found in *Spiraea thunbergii* Sieb. and *Spiraea prunifolia* Sieb. and conjugated form also exert a similar inhibitory effect on the root growth, suggesting that the essential chemical structure responsible for the inhibitory activity is *cis*-CA (Hiradate et al., 2005; Guo et al., 2011). It is reasonable to speculate that the real level of *cis*-CA in plant tissues should be much higher if we combine both the free and conjugated forms. The coumaric acid β-D-glucosyltransferase seems to have a higher specificity for *cis*-CuA than its *trans*-CuA, which suggest that enzymatic system may exist in plant to convert the free *cis*-CA into conjugates (Guo et al., 2011). In addition to conjugates, Guo et al. (2011) reported that other *cis*-phenyl-propenoids like *cis*-CuA, *cis*-FA and *cis*-SA have been shown to increase *ZCE1* transcript accumulation.

cis-CA, *cis*-CuA, *cis*-FA and *cis*-SA were prepared according to the method described by Yang et al. (1999). *trans*-Isomer was dissolved in ethanol and was irradiated by 6–12 h with an UV lamp. After ethanol evaporation the *cis*-isomer was isolated dissolving the irradiated CA in water and sonicated for 5 min. It is stated that the ^{1}H-NMR analysis was used for the structure characterization, but call the attention that any detail is added about the degree of purity of the *cis*-isomers prepared and used in the experiments (Yang et al., 1999). As it is known (see discussion bellow) it is quite difficult to prepare high pure *cis*-cinamic isomers with this protocol. Similarly, other authors suggested the photoisomerization as a method of synthesis but they use HPLC to separate the isomers in order to get the *cis* acids as pure compounds with purity ～100% (Sun et al., 2002; Chen et al., 2005).

Complementary, in order to point how the geometry of the molecule also affect the role of *trans*- and *cis*-cinnamic acids in mammalian cell physiology we can summarize that: The *cis*-CA exerts higher number of physiological activities than *trans*-CA in many aspects (Yen et al., 2011). The *trans*-CA has been investigated extensively for its potential pharmacological effects whereas the study of *cis*-CA is limited because pure *cis*-CA was hard to be obtained. The *cis*-CA develops greater antituberculosis and synergistic effect against multiple-drug resistant tuberculosis (MDR-TB) than *trans*-CA (Chen et al., 2011). Therefore, *cis*-CA might be potential anti-mycobacterial or synergistic agent that can be developed to against tuberculosis (Chen et al., 2011). Also, the *cis*-CA seems to exert higher inhibitory effect against invasion of human adenocarcinoma A549 cells than *trans*-CA (Chen et al., 2011) and the inhibitory effect might result from reducing matrix metalloproteinase MMP-2 (its expression has been associated with the invasion of highly metastatic adenocarcinoma A549 cells) and MMP-9 (its secretion has been reported in lung, colon, and breast cancer) activities and prohibiting migratory capability of the cells (Yen et al., 2011). The results from *in vitro* studies of the metabolism of *cis*-CA by rat liver cell-free homogenate with 4-pentenoic acid suggest that this isomerization reaction is the first step in the metabolism of *cis*-CA by rat liver (Sun, 2003). This finding suggests that an isomerase is constitutively present in rat liver to catalyze the transformation reaction of *cis*-CA to *trans*-CA. The *trans*-CA formed from *cis*-CA was then catabolized by a cycle of beta-oxidation to form benzoyl-CoA. More than 75% of this resultant benzoyl-CoA was conjugated with glycine to produce hippuric acid, suggesting this pathway is the major route for rat liver to catabolize *cis*-CA. A pathway for the metabolism of *cis*-CA in rat liver is proposed based on this data (Sun, 2003).

cis-cinnamic acids role in plant physiology

Cause of limitation of the studies in vivo

As we discussed previously cinnamic acids exists in both *cis*- and *trans*-forms in the nature. Nowadays, in the area of food chemistry, analytical biochemistry and foodomics (Shahadi and Naczk, 2004; Herrero et al., 2012), the interest in both cinnamic acids isomers has increased dramatically because they were detected in all kinds of plant derived products: i.e., foods, herbs medicines and cosmetics (Caccamese et al., 1979; Taiz and Zeiger, 2006). Although the two forms have been found to exist in the plant cells and they have shown to be bio-active, experiments with the *cis* form are not performed because it is not commercially available and it is quite difficult the isolation of enough amount of a very high pure *cis* isomer from the *trans/cis* natural mixtures extracted from plant tissues or obtained by photoisomerization. Few reports in literature try to get them and achieved this aim successfully, obtaining each isomer separated efficiently. As it has been discussed in the previous section, the *trans*-isomers of cinnamic acids serve as precursors for the biosynthesis of phenolic compounds such as flavonoids, phytoalexin, salysilic acid and lignin. As was also previously mentioned even their role in plant and mammal cells has been extensively studied because all of them are commercially available. In contrast, only the synthetic *cis* isomer of cinnamic acid (*cis*-CA), which within this metabolites family is the only *cis*-cinammic commercially available, has been studied in some extent.

The biosynthesis of *trans* isomers is quite well established (Figs. 1–6). On the contrary for the *cis* forms speculative pathways are still under discussion (Fig. 7). As we

previously discussed, it has shown they have cell elongation-promoting activity based on several bioassays, inhibit root growth of avena (*Avena sativa*), wheat (*Tritiucum aestivium* L.), flax (*Linum usitatissimum*) and *Arabidopsis thaliana* and cause epinastic curvature in tomato seedlings (*Lycopersicon esculentum* Mill.) like plant hormones, i.e., auxin and ethylene (Wong et al., 2005). To explore the possible physiological roles played by *cis*-CA and other *cis*-cinanmic acid derivatives found in nature (plant tissues) (see Table 1 and Figs. 1–5) it is essential to have these *cis* isomers commercially available and/or simple and friendly protocols for its preparation-isolation and purification in the laboratory. As states Sun et al. (2002) due to the stability and purity of the unavailable *cis*-cinnamic acids, the mechanism of *cis*-cinnamic acids effects on nutritional, toxicological, and metabolic pathway in eucaryotic cells are virtually unknown. He also adds that as *cis*-isomers of cinnamic acids are in general obtained throught protocols that, as it has been reported in the literature, yield the *cis* species with a significant amount of *trans* isomer as impurity. Then, this may be the reason why the industrial production of *cis*-cinnamic acids has not been transformed in commercialized so far.

cis-cinnamic acids synthesis in vitro

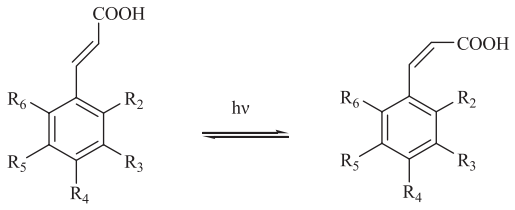
In the literature, there are two ways described to obtain the *cis*-isomers of cinnamic acid family *in vitro*. One is the thermal synthesis (Rao and Filler, 1976) and the other is the photoisomerization from *trans*-isomer with UV light (Arai, 1999; Mori and Inoue, 2005). The first includes drastic, high pollutant and unpractical conditions, while the latter, separation of the desired product is quite difficult.

Briefly, as example of the first group *cis*-CA acid was prepared by isomerization of *trans*-CA acid using polyphosphoric acid (Rao and Filler, 1976); Hydrogenation of phenylpropiolic acid with lindlar catalyst in hexane was carried out to prepare *cis*-CA (Robinson and Cambell, 1933; Chaloner, 1980; Lee et al., 1994; Hanai et al., 2001). An alternative method of synthesis of *cis*-CA by hydrogenation was from a solution of ethyl phenylpropiolate, lindlar catalyst and quinoline, which was warmed and flushed with hydrogen (Reed et al., 1993). The ester produced, was then refluxed in a mixture of ethanol-NaOH, the solution was acidified and finally extracted with organic solvents. Alkaline treatment (NaOH) at room temperature of *trans*-SA, *trans*-FA and *trans*-CuA, was conducted yielding the mixture of *cis* and *trans* isomers (Krygier et al., 1982; Jung et al., 2002). All the examples mentioned afforded the *cis*-isomer with low yield and high contamination.

cis-cinnamic acids synthesis

trans-cis Photoisomerization

Photochemical *trans-cis* (or *cis-trans*) isomerization (Fig. 8) is a major area of interest in modern photochemical research and is also studied, as a special tool for synthesis, in preparative organic photochemistry (Arai, 1999; Rao, 1999; Mori and Inoue, 2005). Photochemical *cis-trans* isomerization has a key role in many photobiological phenomena, such as vision (rhodopsin), ATP synthesis (bacteriorhodopsin), phototaxis (Chlamydomonas), and



trans-CH *cis*-CH

Fig. 8 *trans-cis* Photoisomerization of cinnamic acids. (Arai, 1999; Mori and Inoue, 2005; Turro et al., 2009; Salum et al., 2010).

other allied processes (Mori and Inoue, 2005). It has practical application in industry (Braun et al., 1991; Ullmann's Encyclopedia of Industrial Chemistry, 1991; Kirk-Othmer, 1996), i.e., vitamin A and D processes. Furthermore, it is a likely candidate for many optoelectrical, optomechanical switching, storage devices and light-driven chiroptical molecular motors (Durr, 1990; Klessinger and Michl, 1995; Feringa et al., 2000; Nieuwendaal et al., 2008; Turro et al., 2009). Photoisomerization presents the only direct method for contra-thermodynamic *trans-cis* isomerization of olefins. Synthetic applications of this method have been limited by its reversible nature, which, in the absence of other reactions, leads to a photostationary-state mixture of isomers (Klessinger and Michl, 1995; Rao, 1999; Turro et al., 2009). The composition of these mixtures is governed by isomerization quantum yields and extinction coefficients (direct irradiation) or rate constants for triplet energy transfer (triplet sensitization). Photostationary states enriched in the thermodynamically less stable *cis* isomer are observed only in cases where the trans isomer is more strongly absorbing than the *cis* isomer (i.e., long-wavelength irradiation of the stilbenes) or the triplet energy of the trans isomer is lower than that of the *cis* isomer (i.e., triplet-sensitized isomerization of β-ionols).

Unfortunately, neither direct irradiation nor triplet sensitization provides a general method of selective *trans-cis* isomerization. In addition to triplet sensitization, various additives (radical initiators: electron acceptors, protic acids, metal ions, metal carbonyl complex, lanthanide shift reagents, etc.) and restricted environments (high pressure, organized assemblies, solid state, etc.) have been used to alter photoisomerization pathways. However, none of these methods induce high-yield *trans-cis* conversion (Arai, 1999; Rao, 1999).

There are few citations for the photochemical *trans* to *cis* one way isomerization process. i.e., photoisomerization process in furyl- and imidazole- substituted ethylkenes (Mori and Inoue, 2005). The direct excitation of these compounds in solution leaded to *trans* to *cis* isomerization with almost 1:1 photostationary state composition. The same reaction was conducted in the presence of Lewis acid (Lewis acid-olefin complex), which shows a high preference for the formation of the *cis* isomer (% yield 88–94) although the *trans* isomer (6–12 %) as contaminant is present in the isolated product.

There are various factors governing this process. The effect of wavelength, conformation, configuration, and

other factors was taken into account to highlight the one way *trans* to *cis* isomerization. It was presumed that hydrogen bonding plays an important role in the one way *trans* to *cis* isomerization.

One-pot cis-cinnamic acids synthesis by photoisomerization of ionic liquid trans-cinnamic acids

Room-temperature ionic liquids (ILs) are useful as solvents for organic synthesis, electrochemical studies, and separations. They possess negligible vapor pressure, are non-explosive, non-flammable and are stable at temperatures below 250°C. They are nano-structurated compounds whose properties can be tuned by variation of the cation and anion nature. There are countless combinations possible, which make ILs well suited to creating tailor-made or "designed compounds" with different densities, viscosities, melting (decomposition) point, polarity, optical properties, etc. (Wasserscheid et al., 2002). Particularly, those formed by a crystalline organic acid MALDI (matrix assisted laser desorption ionization) matrix compound as the anion and a protonated organic base as the cation are potential new MALDI matrixes (ILMs) of use in MALDI MS (mass spectrometry). They will keep the peculiar characteristics of the ILs described above (Armstrong et al., 2001; Tholey and Heinzle, 2006).

Although the ILs typical properties above mentioned are quite proper for new ILMs, their photochemical and thermal stability can not be predict *a priori* without concrete photochemical and thermal experiments.

As part of a project related with the study of the thermal and photochemical properties of current used organic crystalline MALDI matrixes (Mesaros et al., 2006; Tarzi et al., 2009; Cole, 2010; Petroselli et al., 2012) in order to improve MALDI MS analysis and to develop new matrices (Nonami et al., 1997; Nonami et al., 1998; Landoni et al., 2008; Gholipour et al., 2010) several ILs were prepared combining commercially available *trans*-cinnamic acids (*trans*-CH) (Fig. 9, i.e., *trans*-FA) and an organic bases (Am) (Fig. 9, i.e., Am=butylamine) (Salum and Erra-Balsells, 2009; Salum et al., 2010). In this project, the photostability of *trans*-cinnamic IL was studied in acetonitrile solution.

For our surprise and good luck, we found out a one-pot method for the preparation-purification-isolation as 100% pure *cis* isomer without any additional chemical manipulation for purification of the *cis*-cinnamic IL; it further leads to the corresponding *cis*-cinnamic acid easily, just pH adjusting the *cis*-cinnamic IL methanol solution. Thus we reported for the first time a highly efficient one-pot preparation-isolation-purification process of *cis*-cinnamic acids by photoisomerization in solution of *trans*-cinnamic acids IL. Note in Fig. 10 the notorious different geometry shown by the pair *trans* (left) and *cis* (right) isomers of SA as the ionic liquids with ethanolamine.

We hypothesized that the end terminal structure of the amine would approach the aromatic (phenyl) moiety of the cinnamic acid structure in a synclinal overlapping fashion to provide the required distance among functional groups to generate stabilizing intramolecular (intra ionic liquid molecule) interactions such as hydrogen bridge or hydrophobic/

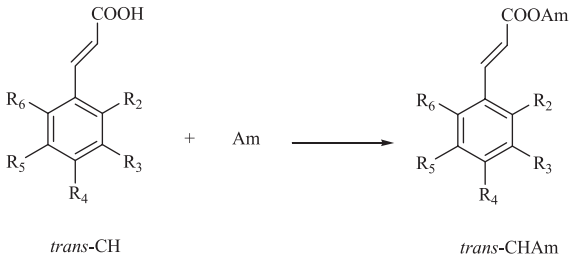


Fig. 9 Ionic liquid preparation with *trans*-cinnamic acid+amine (*trans*-CH+Am). (Salum and Erra-Balsells, 2009; Salum et al., 2010).

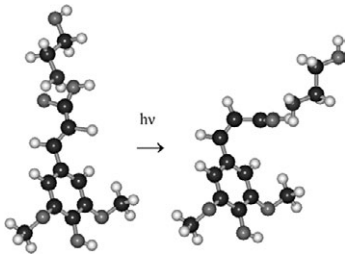


Fig. 10 *trans*-cis Photoisomerization of ionic liquid cinnamic acids (*trans*-CHAm to *cis*-HAm). As example *trans*-SA. Am to *cis*-SA. Am, with Am=ethanolamine. Adapted with permission from Salum et al. (2010) © Copyright 2010 American Chemical Society.

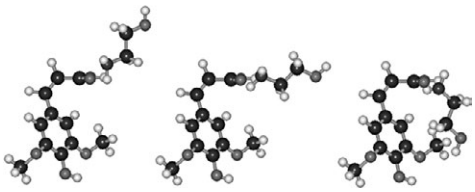


Fig. 11 Different tridimensional structures of the *cis* ionic liquids; i.e. cis-SA. Am, with Am=ethanolamine. Adapted with permission from Salum et al. (2010) © Copyright 2010 American Chemical Society.

hydrophobic interactions (Fig. 11). Note the peculiar intramolecular interaction that is possible because of the flexibility and the special geometries that can be adopted by the *cis*-isomer ionic liquids. This special super-folded structure would be the cause of the minimum solubility and exclusive precipitation of the pure *cis*-isomer species.

CONCLUSIONS

Research connected with the study of the role of *cis*-cinnamic acids in nature as well as its effects on growing of plant organs and on health of living animals, insects and humans that are in contact with them, is still quite obscure. The main reason is just because shortage of appropriate methods to prepare, at low cost and with high purity, *cis*-cinnamic acids. The recent introduction of a very simple one-pot methods based on the well known *trans-cis* photoisomerization of the cinnamic moiety but using ionic liquid *trans*-cinnamic derivatives will allow researchers to prepare easily in their laboratory the desired *cis*-cinnamic acid with high purity and ready to be used.

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