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Chiral Inversion of Active Compounds in Plant Extract

Ngoc-Van Thi Nguyen

Abstract

Chiral inversion is always mediated by enzymes and varies with solvent, pH and temperature. Considerable attention should be paid to the mechanism of the inversion reaction and its pharmacological and toxicological results. This chapter will discuss the mechanism of chiral inversion of plants in secondary metabolize and its importance in creating pharmacology consequences. Plant stereoisomers of alkaloids and flavonoids exhibit a wide range of pharmacological effects. Recent advances in chiral analysis for the herbal plants in clinical research & forensic toxicology by experiments in which one enantiomer was given to the experiment subjects in a specific situation. Demonstration of metabolic chiral inversion may have consequences for the development of a new pharmaceutical entity. Hence, it helps a better understanding of chiral compounds in plants, facilitating the application for drug development from medicinal herbs and thereby reducing bioanalytical and toxicology workload.

Keywords: chiral inversion of plants, eutomer, distomer, racemization

1. Introduction

Chiral inversion is the process by which enzymes modify the three-dimensional structure of a molecule by converting one enantiomer to its antipode [1]. Racemization occurs when isomerization leads in the creation of a racemic mixture. As a result, chiral inversion influences drug stability throughout drug discovery and development. Biological activity, toxicity, shelf-life and dosage of the compound are affected by the stability of the drug [2]. The process of chiral inversion is affected by a lot of variables, consequently, the strength of chiral inversion under different situations and in various substances can vary significantly. The primary elements that were acknowledged to play a vital part in the process of chiral inversion were reported to be interspecies differences and tissue types. Some recent researches have demonstrated that additional variables, such as administration route or interaction with other xenobiotics, can also impact enantiomeric conversion.

Plants create a vast diversity of physiologically active metabolites, many of which have stereochemical variants on the same molecular scaffold. These alterations in stereochemistry have a significant influence on biological function. Notably, plant stereoisomers of alkaloids and flavonoids exhibit a wide range of pharmacological effects. Alkaloids are cyclic chemical molecules with a negative oxidation state of nitrogen. They are found throughout the flora and play an important function in plant

protection, sprouting, and encouraging plant development. Plants containing alkaloids are frequently employed as traditional remedies, and these chemicals typically have specific pharmacological actions. The majority of alkaloids are in a chiral form which is often appeared in products as racemic compounds, while their enantiomers have been proved to have different pharmacological actions [3]. Flavonoids are a vast category of polyphenolic chemicals with a benzo—pyrone structure that is found in all plants. Phenylpropanoid is their produce's pathway. Recent interest in these chemicals has been sparked because of the possible health advantages of these antioxidant polyphenolic compounds [4]. The relevance of racemic flavanones stereospecific pomological disposition has been determined and described in the last 20 years. The majority of these studies report on the measurement of flavanones in citrus fruit juices and herbs [5].

Can all chiral compounds undergo chiral inversion? Maybe no, many compounds still can be considered stable in metabolize process. Why are some enantiomers of plants inverted by enzymes and others are not attacked? The reason lies in the structure. The intent of this chapter is to provide a comprehensive, rather than an exhaustive, appraisal of chiral bio-inversion. This chapter will discuss enzymatic chiral inversion of plants in secondary metabolize and its importance create pharmacology effect. Therefrom, it helps a better understanding of chiral compounds in plants, facilitating the application for drug development from medicinal herbs.

2. Mechanism of inversion

Under selective conditions, racemization or enantiomerization defined as the chiral conversion of enantiomer into its antipode may present in many plants metabolizing. When the chiral molecule enantiomers in herbals interact with a chiral macromolecule-like enzyme, they generate a pair of diastereoisomeric complexes that vary energetically. It is not surprising, then, that the results of enzyme-mediated reactions performed on a pair of enantiomers may differ in type and/or extent. Indeed, given the structure of the enzyme-substrate complex, it is plausible to believe that enantioselectivity is the rule rather than the exception in metabolism. Likewise, the binding of a prochiral substrate to an enzyme may orient two enantiotopic groups differently about the enzyme catalytic site, causing these two groups to become diastereotopic within the enzyme-substrate complex. It's simple to see how the production of a chiral metabolite from a prochiral substrate may result in stereoselectivity for one isomeric product [6].

At the substrate and product levels, xenobiotic metabolic reactions exhibit two forms of stereoselectivity. As a result, they can be classed according to their stereoselectivity or, if such selectivity is complete, their stereospecificity. Caution while using this latter phrase, because the ability to determination "specificity" is clearly dependent on the analytical approach of the research. The words substrate and product "stereospecificity" were initially introduced to the enzyme-mediated reduction of ketones by Prelog [7], and were later extended to drug metabolic processes by Jenner and Testa [8]. Substrate stereoselectivity is the preferred metabolism of one of two stereoisomers over the other, whereas product stereoselectivity is the preferential production of one stereoisomer over the other stereoisomers that may exist. These two "selectivities" may be so closely related that substrate-product stereoselectivity, i.e., the selective metabolism of one of a pair of enantiomers to form one of several possibly diastereoisomeric products, may also be seen. If the enantiomeric

composition of medication or metabolite is detected in the analysis process, data collected from in vivo research on stereochemistry in plants must be regarded with caution (Table 1).

2.1 Alkaloids

Plants are thought to generate over 12,000 distinct alkaloids, which may be classified based on their carbon skeleton structures. Many catalytic stages in alkaloid biosynthesis in plants are catalyzed by enzymes from various protein families.

Since the discovery of morphine in 1806, the complex relationships between opium poppy and the human condition have fueled substantial study into the production of morphinan alkaloids [23]. During the 1960s, significant progress toward route elucidation was made, which supported a major theory [24] that morphine was

Plant	Enzyme	Stereoselective	Stereospecificity	Ref.
Alkaloid Compound				
Opium poppy	1,2-dehydroreticuline reductase		(R)-reticuline	[9]
Catharanthus roseus	tetrahydroalstonine synthase		(3S,19S,20S)-Tetrahydroalstonine	[10]
Claviceps purpurea	Dimethylallyl tryptophan synthase	L-tryptophan		[11]
Hyoscyamus niger	Hyoscyamine 6β-hydroxylase	L-hyoscyamine		[12]
Berberis koetianeana	Tetrahydrobenzyliso-quinoline- <i>N</i> -methyltransferase		(<i>R</i>)-tetrahydropapaverine	[13]
Flavonoid Compound				
Soybean	Chalcone isomerase		(2S)-flavanone	[14]
Dahlia variabilis	Flavanone 4-reductase	(2S)-flavanone	(2S, 4R)-flavan-4-ol	[15]
Citrus unshiu	Flavonol synthase	(2R,3R)-dihydroflavonol		[16]
Glycyrrhiza echinata	Flavanone 2-hydroxylase	(2S)-flavanone		[17]
Ginkgo biloba Pseudotsuga menziesii	Dihydroflavonol 4-reductase	(2R,3R)-dihydroflavonol	(2R, 3S, 4S)-flavan-2,3-trans-3,4-cis-diol	[18]
Medicago truncatula	Anthocyanidin reductase		(2R, 3R)-flavan-3-ol	[19]
Medicago sativa	Isoflavone reductase		(2R)-isoflavanone	[20]
Pisum sativum	Hydroxymaackiain-3-Omethyltransferase	(+)-6a-hydroxymaackiain		[21]
Desmodium uncinatum	Leucoanthocyanidin 4-reductase	flavan-2,3-trans-3,4-cisdiol	(2R, 3S)-flavan-3-ol	[22]

Table 1.
Stereoselective and/or specific enzymes of alkaloid and flavonoid compound biosynthesis in plant extract.

generated by 1-benzylisoquinoline alkaloid metabolism [25]. Because only the (R)-conformer could undergo additional phenol coupling to the morphinan scaffold, (S)-reticuline emerged as the primary 1-benzylisoquinoline intermediate, with its stereochemical inversion to (R)-reticuline thought to be a critical gateway reaction [26].

The pathway makes use of opium poppy reticuline epimerase, a multi-domain protein composed of the P450 CYP82Y2 linked to an aldo-keto reductase (AKR). CYP82Y2 (1,2-dehydroreticuline synthase, DRS) catalyzes the conversion of (S)-reticuline to 1,2-dehydroreticuline, which is then converted to (R)-reticuline by the AKR module (1,2-dehydroreticuline reductase, DRR) [26]. A second P450 called CYP719B1 then transforms (R)-reticuline into salutaridine [27, 28]. This procedure includes (R)-reticuline twisting, reorientating and oxidative C–C bond coupling stimulated by CYP719B1 (**Figure 1**).

Catharanthus roseus, a medicinal plant, creates three of these isomers: ajmalicine (raubasine), tetrahydroalstonine, and 19-epi-ajmalicine (mayumbine) (**Figure 2**) [30]. These heteroyohimbines are produced from deglycosylated strictosidine (strictosidine aglycone), as are the bulk of monoterpene indole alkaloids [31]. A glucose unit removal from strictosidine by strictosidine glucosidase (SGD) leads to the formation of a reactive dialdehyde intermediate that can rearrange to generate a variety of isomers [32]. The stability of these isomers by enzyme-catalyzed reduction is thought to be the first step toward the vast chemical variety found in monoterpene indole alkaloids. The tetrahydroalstonine synthase (THAS) is a zinc-dependent medium-chain dehydrogenase/reductase (MDR) that manufactures the heteroyohimbine tetrahydroalstonine (**Figure 2**) [33]. Although, these studies showed that THAS is an important enzyme for the heteroyohimbine production, the mechanism by which this enzyme controls the stereoselectivity of the reduction remained unexplained. Moreover, the fact that strictosidine aglycone is also a prodrug of some alkaloid scaffolds so constitutes a major branch point in the monoterpene indole alkaloid biosynthesis process [29].

2.2 Flavonoids

Most flavonoid biosynthesis enzymes are extremely stereoselective and/or stereospecific; nonetheless, this assertion is based on just one or a few published findings for numerous enzymes. Flavonoids are produced by the phenylpropanoid pathway, which begins with the enzyme L-phenylalanine ammonia-lyase deamination of phenylalanine (PAL). D-phenylalanine is not a substrate for PAL; it is selective for the naturally occurring L-isomer of phenylalanine [34]. The process mediated by chalcone–flavanone isomerase (CHI), which sets the stereochemistry at C-2 of the flavonoid heterocyclic ring, maybe the most stereo-chemically crucial in flavonoid biosynthesis. CHI is a chemically and structurally well-characterized enzyme that creates (2S)-flavanones from chalcones (**Figure 3**) [14, 35].

Unlike other flavonoid enzymes such as PAL or CHI, the 2-oxoglutarate-dependent dioxygenases flavonol synthase (FLS) and anthocyanidin synthase (ANS) have wide substrate and product selectivities in vitro (both take flavanone, dihydroflavonol, and leucoanthocyanidin as substrates). Prescott et al. have reported a detailed structural and in vitro research on recombinant flavonol synthase from *Arabidopsis thaliana*, with a focus on the stereochemistry of substrate and product, have provided information on how they catalyze reactions with their real substrates in vivo [36]. FLS and ANS prefer substrates with natural C-2 and C-3 stereochemistry [(i.e. (2R,3R)- dihydroquercetin for FLS and (2R,3S, 4R/S)- leucoanthocyanin for ANS], but hydroxylate both (2R)- and

Figure 1.
Proposed chiral inversion of (*S*)-reticulon to (*R*)-reticulon catalyzed by 1,2-dehydroreticulon reductase (DRR) and 1,2-dehydroreticulon reductase (DRR) in opium poppy [9].

Figure 2.
Heteroyohimbine alkaloid biosynthesis. Red highlighted compounds indicate the three diastereomers identified in *Catharanthus roseus*. Alkaloids derived from heteroyohimbines are also illustrated [29].

Figure 3.

General outline of the flavonoid pathway (PAL: Phenylalanine ammonia-lyase, CHS: Chalcone synthase, CHI: Chalcone isomerase, FHT: Flavanone 3 β -hydroxylase, FNS I: Flavone synthase I, FLS: Flavonols synthase, DFR: Dihydroflavonols reductase, ANS: Anthocyanidin synthase). Chiral inversion in flavonoid metabolizes was highlighted by red frame.

(2S)-naringenin equally well in vitro, indicating that the C-3 hydroxyl group is important in biasing substrate selectivity [37].

The flavan-3-ols (+)-catechin and (–)-epicatechin serve as the foundation for proanthocyanidins (condensed tannins), a family of molecules of great interest due to their effects on animal health [38]. The C-2 and C-3 stereochemistries of (+)-catechin (2,3-trans) are identical to those of flavonoid pathway intermediates, and a pathway leading from (2R, 3S, 4S)-leucoanthocyanidin to (+)-catechin, catalyzed by leucoanthocyanidin reductase (LAR), has been illustrated and affirmed by the cloning of a leucoanthocyanidin reductase [22]. LAR belongs to the Reductase–Epimerase–Dehydrogenase protein family, which also includes isoflavone reductase and similar homologs (**Figure 4**) [39].

The process catalyzed by anthocyanidin reductase (ANS) and anthocyanidin reductase (ANR) leads from leucocyanidin to (–)-epicatechin [40]. By operating on an achiral intermediate, ANR, an enzyme with limited sequence similarity to dihydroflavonol reductase, can introduce the 2,3-cis stereochemistry (anthocyanidin). Mechanisms for this reaction have been hypothesized, and it is plausible that more ANR-like enzymes with the potential to introduce different stereochemistries exist (**Figure 5**) [41].

3. Factors affecting chiral inversion

Chiral inversion is always mediated by enzymes and varies with solvent, pH and temperature. When a molecule has two or more elements of chirality, one of which is configurationally labile, enantiomerization can occur. Many studies have been

Figure 4.
 The pro-anthocyanidin pathway showing the LAR reaction.

reported about the chiral compounds inversion such as: evodiamine in *Evodia rutaecarpa* [42], ephedrine and atropine (**Figures 6** and **7**) [43].

Chiral inversion is always mediated by enzymes. One of the most valuable synthetic features of enzymes is their ability to discriminate between enantiomers of racemic substrates [44]. The ratio of stereoisomers is changed mainly by stereospecificity and stereoselectivity of enzymes transformation. The stereoselectivity and stereospecificity of enzymes change dramatically the ratio of drug enantiomers and metabolites enantiomers in biological systems. The enzyme-mediated chiral inversion can be affected by determining expression, substrate affinity and activity of the enzyme. The difference of species and tissue can be different in the rate of the chiral inversion occurrence as well as of the routes and mechanisms of inversion [2].

On another hand, the development of strategies that improves the stereoselectivity of enzyme-catalyzed resolutions has been researching. Modification of the substrate, recycling of the product and changing of the reaction conditions are the three most common ways. From now, even enzymes with modest stereoselectivity can be used successfully [44]. Configurational stability depends mainly on the structure and the conditions, especially with solvent, pH and temperature [2].

According to Ngoc Van Thi Nguyen et al. (2013) research, extraction conditions are also can affect the enantiomerization while this study investigated the optimization of the extraction procedure, more specifically the solvent, pH and temperature [42].

3.1 Solvent

In the metabolic chiral inversion research, avoiding spontaneous or chemical racemization of enantiomers is one of the important things [2]. The organic solvent

Figure 5.
Pathway for CT biosynthesis placing BAN immediately downstream of ANS.

Figure 6.
Structures of (A): (1R,2S)-(-)-ephedrine and (1S,2R)-(+)-ephedrine; (B): (S)-(-)- hyoscyamine and (R)-(+)-hyoscyamine.

Figure 7.
Chemical structure of (1a) R-(-)-evodiamine, (1b) S-(+) evodiamine.

characterism is one other parameter that can significantly interfere with this chiral inversion [45]. The study of Yang SK [46] has shown that racemization half-lives $t_{1/2}$ of enantiomeric oxazepam were 4.8 min in methanol, while it was 840 min in diethyl ether, and 5000 min in hexane, 4500 min in acetonitrile, etc.

3.2 pH

Based on the result of the study of Glass Amanda M. et al. (2012), the data collectively prove that pH has a minute effect on the chiral inversion rate (Figure 8) [48].

The pH effect on proton extraction to give the enolate-form of CoA-thioester resulting in chiral inversion [47]. Chiral inversion and sufficient emission intensity were observed at basic pH 8 and 8.9, respectively, whereas only little emission was observed under neutral to acidic conditions.

Figure 8.
Time-dependent changes of D-luciferin substrate. Luciferin racemization under various pHs of 150 mM GTA buffer, under ddH₂O, and under a medium was monitored for 12 days. The results are highlighted by colors: pH 5 (purple), pH 6 (blue), pH 7 (orange), pH 8 (wine red), pH 8.5 (green), ddH₂O (pink) and DMEM (brown). Even under acidic to neutral conditions, obvious racemization that could not be ignored for long-term experiments were observed. The best condition for inhibiting racemization to maintain D-luciferin optical purity was dissolution in ddH₂O [47].

Figure 9.
The effect of temperature on enzyme activity [49].

3.3 Temperature

Enzyme activity is also affected by temperature, which can lead to the chiral inversion efficiency. The research of the effect of temperature on enzyme activity showed that the hydrogen peroxidase activity's best temperature is 41°C. When this condition is decreased to 37° C, the enzyme activity decreased. Continuing to decrease to 9°C can decrease dramatically the activity of the enzyme. The influence on enzyme flexibility is because of the temperature effect on hydrogen bonds and covalent (Figure 9) [49].

4. Pharmacological consequences

One of the three majorities of racemic pharmaceuticals are the racemic drugs that only have one eutomer, but the distomer could be transformed into its bioactive antipode by chiral inversion in the body (Table 2) [60].

4.1 Alkaloids

Based on many studies about unnatural alkaloid enantiomers, and the results reviewed here the pharmacological effect of natural isomers is enantioselective. However, unnatural enantiomers also have a pharmacological effect of their own which can be discovered in the future. Morphinans of the unnatural (+)-series, in contrast to the (–)-series which are chemically connected with natural morphine, were found to be do not have pharmacological effects as analgesics in vivo, instead, presented useful antitussive properties (Figure 10) [62].

(+)- and (–)-spondomine-racemic and dimeric indole alkaloids have been reported in the study of Tian-Yun Jin (2021) [63], especially, (+)-spondomine displayed cytotoxic against the K562 cell line and exhibited Wnt and HIF1. Moreover, all of them were found to be active in promoted angiogenesis and moderate antiinflammation.

Oleracein E (OE) (8,9-dihydroxy-1,5,6,10b-tetrahydro-2H25 pyrrolo[2,1-a] isoquinoline-3-one), an alkaloid possessing tetrahydroisoquinoline and pyrrolidone skeletons. It was reported to have a lot of pharmacological effects such as: anti-bacterial, anti-inflammatory, anti-aging, anti-hypoxia, anti-oxidant, skeleton-relaxant, hypolipidaemic, analgesic, hypoglycemic, cognition-improvement and neuroprotective functions, especially the optical isomer of (+)-oleracein E (OE) called

Plant	Stereoisomer compound	Pharmacological effect	Test model	Ref.
Alkaloids				
Huperzia serrata	Huperzine A and B	Anticholinesterase activity	Acetylcholinesterase (AChE) inhibitory assay	[50]
Narcissus jonquilla quail	Jonquailine	Anticancer	Human cancer cell line: A549; OE21; Hs683 U373;SKMEL B16F10	[51]
Uncaria rhynchophylla	Speciophylline	Antiplasmodial activity	<i>Plasmodium falciparum</i>	[52]
Isatis indigotica	Isatindigotindoline	Inhibitory effects on β -amyloid aggregation	Thioflavin T (ThT)-binding assay	[53]
Flavonoids				
Centaurea maculosa	Trans-flavan-3-ol (+)-catechin	Antibacterial	<i>Xanthomonas campestris</i> , <i>Pseudomonas fluorescens</i> , <i>Erwinia carotovora</i>	[54]
Citrus fruit	Narirutin, naringin, hesperidin and neohesperidin	Antioxidant	DPPH assay	[55]
Psidium guajava	Quercetin-3-O- α -L-arabinopyranoside	anti-Streptococcus mutans activity	<i>S. mutans</i>	[56]
Rhus retinorrhoea	Persicogenin	Anticancer	MCF-7, HeLa, and HT-29 cells	[57]
Silybum marianum	Silibinin A and Silibinin B	Anticancer	MDA-MB-468 breast cancer cells of the control mice	[58]
Leucoscepttrum canum	S-(+)- and R-(-)-leucoflavonines	Aticholinesterase activity	Acetylcholinesterase (AChE) inhibitory assay	[59]

Table 2.
Pharmacologic effect of stereoisomer compound in plant extract.

(–)-trolline has remarkable antibacterial as well as moderate antiviral activity against influenza viruses A and B [64].

4.2 Flavonoids

According to Blair, Lachlan M. (2016) [65], (–)-Foveoglin A (5) exhibited cytotoxicity against a panel of cancer cell lines, while (+)-isofoveoglin (7) and (–)-cyclofoveoglin (8) were weakly cytotoxic, and (+)-foveoglin B (6) was inactive (Figures 11 and 12).

Characterize the stereoselective pharmacokinetics of pinocembrin and pinostrobin and their bioactivity in some in vitro investigation with relevant roles in heart disease, colon cancer, and diabetes etiology and pathophysiology [66]. These investigations have revealed that chiral differences in the chemical structure of these compounds result in significant pharmacodynamic differences. Pinocembrin and pinostrobin demonstrated concentration-dependent alpha amylase inhibitory activity. While pinocembrin also has anti-inflammatory antioxidant in the pure S-enantiomer and racemate.

Figure 10.

Biosynthesis of morphine in plants. * These metabolic conversions are highly stereoselective [61].

Figure 11.

Aglain and aglaforbesin flavoalkaloids 1–7, 10–12 [65].

Figure 12.
Aglain and aglaforbesin flavoalkaloids 8, 9 [65].

Figure 13.
Chemical structure of daidzein (a) and its metabolites (b and c).

Racemic liquiritigenin is proved to be a dose-dependent inhibition of alpha-amylase enzyme whereas its pure enantiomers did not have this bioactivity. Racemic liquiritigenin showed moderate antiproliferative activity on an HT-29 cancer cell line that was also dose-dependent and had inhibitory effects on the cyclooxygenase-2 enzyme [67].

Racemic liquiritigenin, which was dose-dependent, has been proved its moderate antiproliferative activity on a cancer cell line_ HT-29, and inhibitory effects on the cyclooxygenase-2 enzyme [67]. The nature type of naringenin, hesperetin and hesperidin is S - enantiomer, but both R and S enantiomers can have biological activities such as: antitumor, antioxidant and anti-inflammatory [68]. The two enantiomers of equol: R-(+)-equol and S-(−)-equol have been researched in antitumor activity which shown a significant decrease in the number of palpable tumors presented in animals feeding R-(+)-equol compared to the S-(−)-equol's result (Figure 13).

5. Conclusion

Chiral inversion is always mediated by enzymes and varies with solvent, pH and temperature. Considerable attention should be paid to the mechanism of the inversion reaction and its pharmacological and toxicological results. Recent advances in chiral analysis for the herbal plants in clinical research & forensic toxicology by experiments

in which one enantiomer was given to the experiment subjects in a specific situation. Demonstration of metabolic chiral inversion demonstration may give an answer for the development of a new pharmaceutical entity. Understanding more about the factors facilitating such interconversions may considerably aid herbal plant development thanks to this feature determination at an early stage and thereby reducing bioanalytical and toxicology workload.

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Conflict of interest

The authors declare no conflict of interest.

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