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•Review•

Strictosidine synthase, an indispensable enzyme involved in the biosynthesis of terpenoid indole and β-carboline alkaloids

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[ABSTRACT] Terpenoid indole (TIAs) and β-carboline alkaloids (BCAs), such as suppressant reserpine, vasodilatory yohimbine, and antimalarial quinine, are natural compounds derived from strictosidine. These compounds can exert powerful pharmacological effects but be obtained from limited source in nature. the whole biosynthetic pathway of TIAs and BCAs, The Pictet–Spengler reaction catalyzed by strictosidine synthase (STR; EC: 4.3.3.2) is the rate-limiting step. Therefore, it is necessary to investigate their biosynthesis pathways, especially the role of STR, and related findings will support the biosynthetic generation of natural and unnatural compounds. This review summarizes the latest studies concerning the function of STR in TIA and BCA biosynthesis, and illustrates the compounds derived from strictosidine. The substrate specificity of STR based on its structure is also summarized. Proteins that contain six-bladed four-stranded β-propeller folds in many organisms, other than plants, are listed. The presence of these folds may lead to similar functions among organisms. The expression of STR gene can greatly influence the production of many compounds. STR is mainly applied to product various valuable drugs in plant cell suspension culture and biosynthesis in other carriers.

[KEY WORDS] Biosynthesis; Pictet–Spengler reaction; β-Carboline alkaloids; Strictosidine synthase; Terpenoid indole
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Introduction

Terpenoid indole alkaloids (TIAs) are a large group of almost 2 000 natural compounds widely distributed in the families Rubiaceae, Apocynaceae, and Loganiaceae, with structures containing benzene and pyrrole [1]. Some of TIAs are reported to exhibit various pharmacological effects. For example, camptothecin is widely used for cancer treatment [2], and vincristine and vinblastine are valuable antineoplastic drugs [3]. Prescription drugs, such as the central nervous system suppressant reserpine [4], the antihypertensive serpentine [5], the vasodilatory yohimbine [5], and the antimalarial quinine [6], also belong to the TIA group.

Notably, β-carboline alkaloids (BCAs) are the TIAs that connect pyridine to the original indole, which are widely distributed in the plant kingdom with strong pharmacological effects. Several BCAs, such as harmaline, harmine, harmane, and norharmane, remarkably inhibited acetylcholinesterase (AChE) activity; thus, these compounds are regarded as potential anti-Alzheimer drugs [7]. They exert powerful pharmacological effects on the nervous system through interaction with the serotonin, dopamine, benzodiazepine receptors [8, 9], and other neurotransmitter receptors. With respect to their presence in various foods and mammal tissues, organs, and plasma, it is indicated that harmane and other simple BCAs may be endogenous [10, 11]. Furthermore, some BCAs have anti-inflammatory, antitumor, antianxiety, antidepressant, antiplatelet, antiarrhythmic, and antidiabetic properties [12, 13]. TIAs and BCAs are often investigated together in that they share similar chemical structures and synthesis pathways in plants. Moreover, both of them are derived from strictosidine that comes from the Pictet–Spengler reaction (P–S reaction) [14].

The P–S reaction was discovered almost 100 years ago. It is an essential step in the generative process of TIAs and BCAs in natural and organic syntheses [15, 16]. The former mainly occurs in plants and organisms, whereas the latter primarily occurs in man-made environments and usually in-

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cludes organic and acid solvents [14]. BCAs synthesized in organic environments usually require strictly controlled conditions, and the production is barely satisfactory due to the presence of impurities with similar structures to the final product [14, 17]. Compared with organic synthesis, the P–S reaction in plants or other organisms is more efficient and safer. Vincristine is difficult to obtain because of its limited sources. Obtaining this compound from plants and other organisms is easier compared with chemical synthesis [18]. Most notably, the biosynthetic pathways of BCAs and TIAs are catalyzed by the same enzyme strictosidine synthase.

Strictosidine synthase (STR; EC 4.3.3.2) is an indispensable enzyme in plants, and often acts as an important precursor of many alkaloids [19]. STR plays an important role in the production of TIAs and BCAs, achieving high yield. Thus, this enzyme is a vital element in the production of compounds that are difficult to acquire. STR was first isolated from plant cell suspensions of *Catharanthus roseus* [20] and *Rauvolfia serpentina* [21]. Researchers have investigated the influencing factors which increase the production of target alkaloids, such as the external environment, STR gene expression, and other proteins in the reaction. Given that the P–S reaction is a popular method for the syenthesis of many alkaloids, several studies have been conducted to investigate STR structure, reaction mechanism, and biosynthetic engineering [18, 19, 22]. However, the latest summary of STR was conducted about ten years ago and solely focused on its structure [23], while no systematic and comprehensive review about STR characteristics is available.

In the current review, we will summarize the studies concerning the structure of STR and the factors that influence STR gene expression, so as to broaden STR's potential applications, provide reference for future STR studies and enrich our knowledge of proteins and natural compounds. Moreover, new insights into the connection between STR structure and strictosidine derivatives will also be discussed.

Strictosidine-Derived Alkaloids in Plants

The biosynthesis of natural products in plants requires the action of different enzymes. STR catalyzes the P–S reaction between tryptamine and secologanin, leading to the formation of strictosidine, an indispensable intermediate compound during BCA and TIA biosynthetic pathways. Strictosidine can derive to various indoles alkaloids by geraniol-8-hydroxylase (G-8-H), strictosidine glusidase (SG), secologanin synthase (SS) and so on.[18]. It is reported that about 2 000 indoles are derived from strictosidine [24, 25]. However, only about 100 compounds have been definitively proved. Some researcher found that another TIA biosynthetic pathway may exist. For instance, camptothecin, which belongs to TIAs, is derived from strictosidine acid rather than strictosidine [26]. The compounds derived from strictosidine are summarized in Table 1 and Figs. 1–5 according to the structure type of parent nucleus.

Among these compounds, only a few TIAs were reported to be derived from strictosidine [27, 28]. Many biosynthesis pathways in plants have not been thoroughly investigated and the synthetic routes of most natural compounds have not been identified yet. With the deepening of research and the finding of new natural reactions, we may discover more specific biosynthetic pathways of TIAs and reveal new whole synthetic routes.

Structure of STR

Structure and reaction mechanism

According to crystallization and X-ray analyses, the structure of STR in *R. serpentina* is a β-propeller comprising six blades that are radially listed around a central six-fold pseudosymmetry axis [22]. With recent technological advancements, great achievements have been made as to the studies on the structure of STR. Every blade has four β-strands, and the strands are twisted so the first inner strand is nearly perpendicular to the fourth outer strand. Such a structure is the first example of this type found in the plant kingdom [22]. Similar to most β-propeller proteins, STR does not show sequence similarities nor does it share functional homologies with other six-bladed β-propeller proteins [48]. Other STR regions can influenceprotein activity, which is observed through circular permutation [19]. Three helices exist in the STR structure. Two helices are connected by disulfide bridge between residues Cys-89 and Cys-101. Studies demonstrated that the covalently bound cysteine residues play an important role in the integrity of the substrate binding site [22]. The rest helix is located between strands and forms a cap over the active site. The helix shapes the substrate binding pocket and contributes to forming an entrance for substrates [44]. Secondary structure analysis of STR in *Catharanthus roseus* indicated that alpha helix dominated among secondary structure elements through self-optimized predicition method with alignment [49].

Developments in X-ray technology allow researchers to determine the active center of STR which contains about 14 amino acids (Fig. 6) [50]. The binging sites where substrates combine with STR are unequivocal (Fig. 6). Tryptamine is located at the bottom of the molecule, where the amine group is connected with the residue Glu309 by a hydrogen bond. Secologanin binds in the same pocket with its ester group facing toward the bottom, and its site is in the entrance. The hydrophilic glucose unit is outside the pocket, and the aldehyde group of the secologanin directly faces Glu309. Secologanin is close to tryptamine, and the aldehyde group of secologanin is proximal to the amine group of tryptamine.

The structure of the active site indicates that the enzymatic catalytic reaction between tryptamine and secologanin is similar to the chemical reaction that a Schiff base between tryptamine and secologanin initially forms (Fig. 7). And Glu309 may play an essential role in the reaction [51]. Based on the structure mentioned above and the reaction mechanism, hexacyclic-fused cymoside was synthesized by biomimetic P–S reaction [52]. Furthermore, the biocatalytic

Table 1 A summary of compounds derived from strictosidine

Type	No.	Compound	Formula	Plant	Reference
BCAs	**1**	Ajmalicine	$C_{21}H_{24}N_2O_3$	*Rauwolfia serpentina*, *Rauvolfia verticillata*	[29-32]
	2	Antirhine	$C_{19}H_{24}N_2O$	*Catharanthus roseus*	[33]
	3	Cathenamine	$C_{21}H_{22}N_2O_3$	*Catharanthus roseus*, *Rauvolfia verticillata*	[32, 34]
	4	Cinchophyllines	$C_{31}H_{36}N_4O_2$	*Cinchona ledgeriana*, *Cinchona robusta*	[35, 36]
	5	Corynantheal	$C_{19}H_{22}N_2O$	*Cinchona ledgeriana*, *Cinchona robusta*	[35, 36]
	6	Dialdehyde	$C_{21}H_{24}N_2O_4$	*Ligustrum obtusifolium*	[37]
	7	Dolichantoside	$C_{28}H_{36}N_2O_9$	*Uncaria tomentosa*	[38]
	8	Geissoschizine (Corynanthe)	$C_{21}H_{24}N_2O_3$	*Tabernaemontana litoralis*, *Catharanthus roseus*	[28, 33]
	9	Heteroyohimbine	$C_{21}H_{24}N_2O_3$	*Catharanthus roseus*	[33]
	10	Mayumbine/19-epi-ajmalicine	$C_{21}H_{24}N_2O_3$	*Catharanthus roseus*	[39]
	11	Mitragynine	$C_{23}H_{30}N_2O_4$	*Mitragyna speciosa*	[40]
	12	Rauniticine	$C_{21}H_{24}N_2O_3$	*Catharanthus roseus*	[39]
	13	Reserpine	$C_{33}H_{40}N_2O_9$	*Rauwolfia serpentine*, *Catharanthus roseus*	[31, 41]
	14	Rhazine	$C_{21}H_{24}N_2O_3$	*Rhazya stricta*	[42]
	15	Sarpagine	$C_{19}H_{22}N_2O_2$	*Rauwolfia serpentina*	[31]
	16	Strictosamide	$C_{22}H_{22}N_2O_5$	*Ophiorrhiza pumila*	[43]
	17	Strictosidine lactam	$C_{22}H_{22}N_2O_5$	*Rhazya stricta*	[42]
	18	Tetrahydroalstonine	$C_{21}H_{24}N_2O_3$	*Rhazya stricta*, *Catharanthus roseus*	[33, 42]
	19	Vallesiachotamine	$C_{21}H_{22}N_2O_3$	*Catharanthus roseus*	[33]
	20	Vallesiachotamine isomers	$C_{21}H_{24}N_2O_3$	*Rhazya stricta*	[42]
	21	Vincamine	$C_{21}H_{28}N_2O_3$	*Rhazya stricta*, *Catharanthus roseus*	[39, 42]
	22	Vitrosamine	$C_{21}H_{26}N_2O_4$	*Catharanthus roseus*	[33]
	23	Yohimbine	$C_{21}H_{26}N_2O_3$	*Rhazya stricta*, *Rauwolfia serpentina*	[31, 42]
	24	3α-Dihydrocadambine	$C_{27}H_{34}N_2O_{10}$	*Uncaria tomentosa*	[38]
	25	3, 14, 18, 19-Tetrahydroangustine	$C_{20}H_{19}N_3O$	*Strychnos angustflpra*	[30]
Indoline derivatives	**26**	Ajmaline	$C_{20}H_{26}N_2O_2$	*Catharanthus roseus*	[30, 41]
	27	Akuammicine	$C_{21}H_{24}N_2O_2$	*Catharanthus roseus*	[39]
	28	Aspidosperma	$C_{21}H_{24}N_2O_2$	*Tabernaemontana litoralis*	[28]
	29	Deacetylvindoline	$C_{23}H_{30}N_2O_4$	*Catharanthus roseus*	[27]
	30	Leepacine isomers	$C_{22}H_{24}N_2O_3$	*Rhazya stricta*	[42]
	31	Norajmaline	$C_{19}H_{24}N_2O_2$	*Catharanthus roseus*	[30]
	32	Quinamine	$C_{19}H_{24}N_2O_2$	*Cinchona ledgeriana*, *Cinchona robusta*	[35, 36]
	33	Strychnine	$C_{21}H_{22}N_2O_2$	*Catharanthus roseus*, *Saccharomyces cerevisiae*	[39, 44]
	34	Tabersonine	$C_{21}H_{24}N_2O_2$	*Rhazya stricta*, *Catharanthus roseus*	[27, 42]
	35	Vincadifformine	$C_{21}H_{26}N_2O_2$	*Rhazya stricta*	[42]
	36	Vincanine	$C_{21}H_{28}N_2O_3$	*Rhazya stricta*	[42]
	37	Vindoline	$C_{25}H_{32}N_2O_6$	*Catharanthus roseus*	[27, 30, 41]
	38	1, 2-Dihydrovomilenine	$C_{21}H_{24}N_2O_3$	*Camptotheca acuminata*	[45]
	39	16-Hydroxytabersonine	$C_{21}H_{24}N_2O_3$	*Catharanthus roseus*	[27]
	40	16-Methoxytabersonine	$C_{22}H_{26}N_2O_3$	*Catharanthus roseus*	[27]
	41	16-Methoxy-2, 3-dihydro-3-hydroxy-tabersonine	$C_{22}H_{28}N_2O_4$	*Catharanthus roseus*	[27]

Continued

Type	No.	Compound	Formula	Plant	Reference
Indoline derivatives	**42**	17-*O*-Acetylajmaline	$C_{22}H_{28}N_2O_3$	*Camptotheca acuminata*	[45]
	43	17-*O*-Acetylnorajmaline	$C_{21}H_{26}N_2O_3$	*Camptotheca acuminata*	[45]
	44	18-Hydroxypseudovincadifformine (Pseudoaspidosperma)	$C_{21}H_{24}N_2O_3$	*Tabernaemontana litoralis*	[28]
3H-indole derivatives	**45**	Akuammiline	$C_{23}H_{26}N_2O_4$	*Catharanthus roseus*	[39]
	46	Eburenine	$C_{19}H_{24}N_2$	*Rhazya stricta*	[42]
	47	Isoakuammiline	$C_{23}H_{26}N_2O_4$	*Catharanthus roseus*	[39]
	48	Preakuammicine	$C_{21}H_{24}N_2O_3$	*Catharanthus roseus*	[39]
	49	Raucaffricine	$C_{23}H_{24}N_2O_5$	*Rauvolfia serpentina*	[46]
	50	Vinorine	$C_{21}H_{22}N_2O_2$	*Rauvolfia serpentina*	[31]
	51	Vomilenine	$C_{21}H_{22}N_2O_3$	*Rauvolfia serpentina*	[31]
Without indole structure	**52**	Cinchonidine	$C_{19}H_{22}N_2O$	*Cinchona ledgeriana*, *Cinchona robusta*	[35, 36]
	53	Cinchonidinone	$C_{19}H_{20}N_2O$	*Cinchona ledgeriana*, *Cinchona robusta*	[35, 36]
	54	Cinchonine	$C_{19}H_{22}N_2O$	*Cinchona ledgeriana*	[35]
	55	Geisedine	$C_{18}H_{22}N_2O_2$	*Gelsemium sempervirens*	[47]
	56	Gelsemine	$C_{20}H_{24}N_2O_2$	*Gelsemium sempervirens*	[47]
	57	Humantenine	$C_{21}H_{26}N_2O_3$	*Gelsemium sempervirens*	[47]
	58	Oxindole mitraphylline	$C_{21}H_{24}N_2O_4$	*Catharanthus roseus*	[34]
	59	Pumiloside	$C_{22}H_{20}N_2O_6$	*Ophiorrhiza pumila*	[43]
	60	Quinidine	$C_{20}H_{24}N_2O_2$	*Cinchona ledgeriana*, *Cinchona robusta*	[35, 36]
	61	Quinidinone	$C_{20}H_{22}N_2O_2$	*Cinchona ledgeriana*, *Cinchona robusta*	[35, 36]
	62	Quinine	$C_{20}H_{24}N_2O_2$	*Cinchona ledgeriana*	[30, 35]
	63	3(*S*)-Deoxypumiloside	$C_{22}H_{20}N_2O_5$	*Ophiorrhiza pumila*	[43]
Other indoles	**64**	Catharanthine	$C_{21}H_{24}N_2O_2$	*Catharanthus roseus*	[27, 41]
	65	Cinchonamine	$C_{19}H_{24}N_2O$	*Cinchona ledgeriana*, *Cinchona robusta*	[35, 36]
	66	Cinchonaminal	$C_{19}H_{22}N_2O$	*Cinchona ledgeriana*, *Cinchona robusta*	[35, 36]
	67	Coronaridine	$C_{21}H_{26}N_2O_2$	*Tabernaemontana litoralis*	[28]
	68	Dihydrosecodine	$C_{21}H_{28}N_2O_2$	*Rhazya stricta*	[42]
	69	Heyneanine	$C_{21}H_{26}N_2O_3$	*Tabernaemontana litoralis*	[28]
	70	Ibogaine	$C_{20}H_{28}N_2O$	*Saccharomyces cerevisiae*	[44]
	71	Polyneuridine aldehyde	$C_{21}H_{22}N_2O_3$	*Camptotheca acuminata*	[45]
	72	Tetrahydrosecodine	$C_{21}H_{30}N_2O_2$	*Rhazya stricta*	[42]
	73	Tetrahydrosecodinol	$C_{21}H_{30}N_2O_3$	*Rhazya stricta*	[42]
	74	Vinblastine	$C_{46}H_{60}N_4O_9$	*Catharanthus roseus*	[39]
	75	Vincaleukoblastine	$C_{46}H_{60}N_4O_9$	*Catharanthus roseus*	[41]
	76	Vincristine	$C_{46}H_{58}N_4O_{10}$	*Catharanthus roseus*	[39, 41]
	77	Stemmadenine (Strychnos)	$C_{21}H_{26}N_2O_3$	*Tabernaemontana litoralis*	[28]
	78	3′, 4′-Anhydrovinblastine	$C_{44}H_{56}N_4O_6$	*Catharanthus roseus*	[27]
	79	3, 19-Oxidocoronaridine	$C_{21}H_{24}N_2O_3$	*Tabernaemontana litoralis*	[28]
	80	16-Epi-vellosimine	$C_{19}H_{20}N_2O$	*Camptotheca acuminata*	[45]

property of STR was utilized to enantioselectively synthesize a series of BCAs and TIAs with potentially meaningful bioactivity [30, 53]. (−)-Strictosidine was produced through the catalysis of STR and took tryptamine and (−)-secologanin as substrates [54]. Researchers heterologously overexpressed STR to enhance the levels of camptothecin [55].

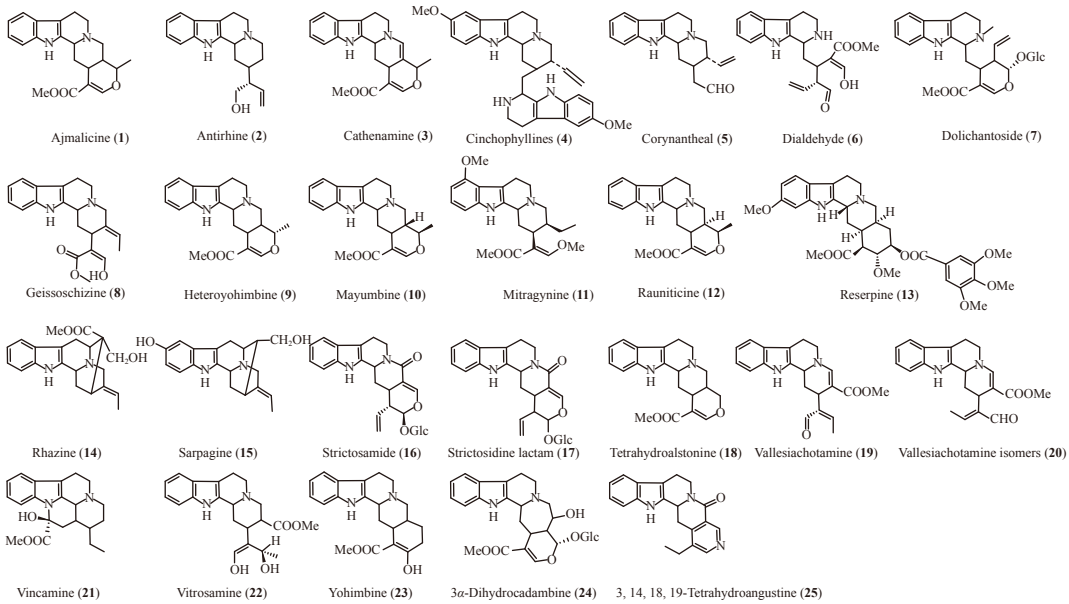


Fig. 1 Representative structures of BCAs

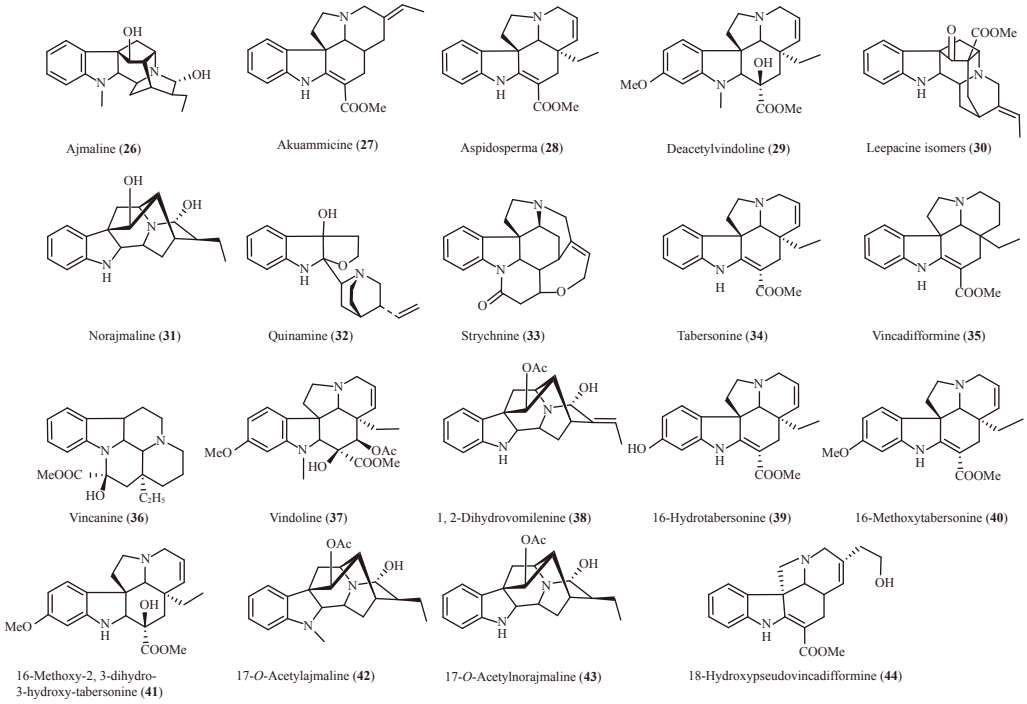


Fig. 2 Representative structures of indoline derivatives

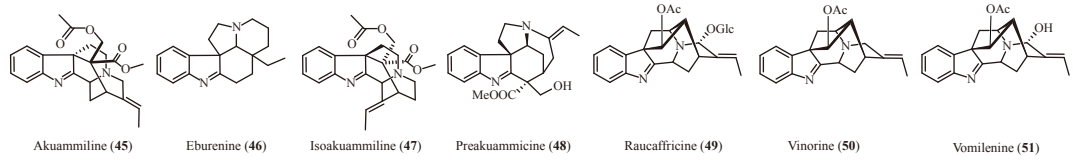


Fig. 3 Representative structures of 3H-indole derivatives

Substrate specificity

The P–S reaction can be regarded as a special type of Mannich reaction that condenses an amine and a carbonyl compound [20]. Many studies have been conducted on the substrate specificity of STR from various plants. These studies show that STR exhibits high substrate specificity that only accepts a few substrates. The present review focuses on STR rather than other isoenzymes and mutations (Tables 2–5).

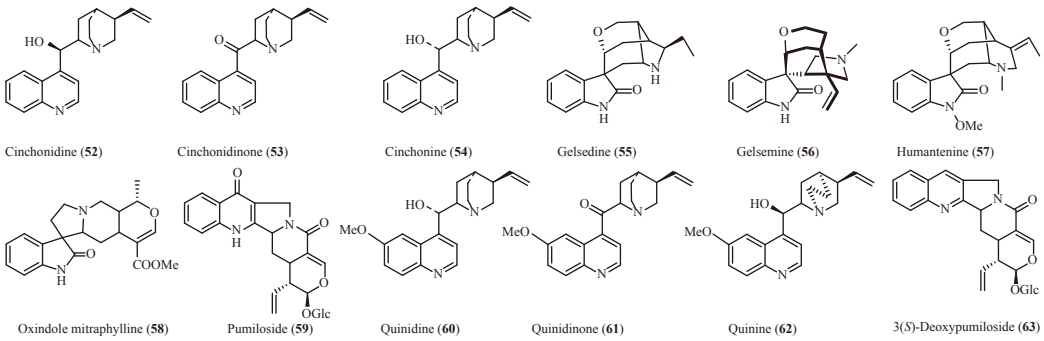


Fig. 4 Representative structures of TIAs without indole structure

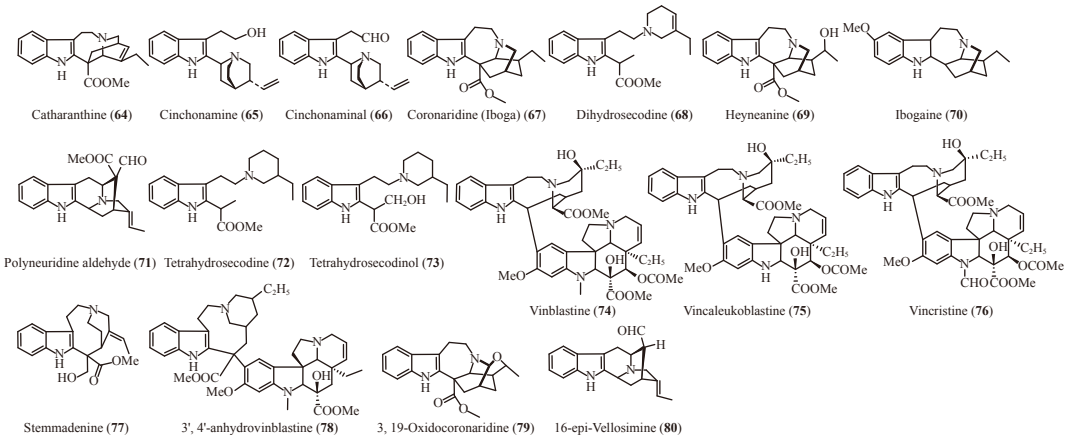


Fig. 5 Representative structures of other indoles

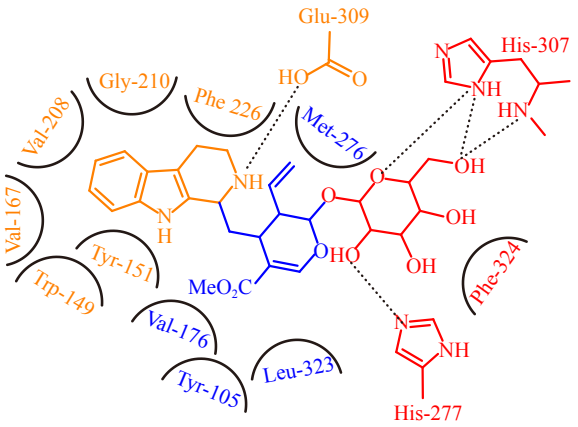


Fig. 6 2D representation of the STR-strictosidine complex [50]

STRs from different plants have different responses to different substrates as each plant has a distinctive gene code [56]. Aldehyde shows high substrate specificity; as long as the vinyl position connects to a big group, STR can not catalyze the reaction [20]. The ester group seems to have endurance to secologanin derivatives [57]. For small-molecule aldehydes, different sources of enzymes lead to opposite results [58, 59]. The side chain exerts a considerable role in the reaction and forms a six-membered ring in the next step, so the productions may come to nothing if the side chain is extremely long, short, or large [20, 56, 57]. The nitrogen atom in the five-membered ring is important and cannot be saturated and become a tertiary amine [22]. Tryptophan and its derivatives can not react with secologanin because of strong electron attraction of carboxyl [22, 50]. STR can catalyze the reaction if the oxygen atom or the sulfur atom replaces the nitrogen atom in the five-membered ring because of their electron donor action [22, 50]. To sum up, both aldehyde and amine show substrate specificity. All substrate features contribute to stable structure of the product. Substrate specificity is important in investigating the structure of STR and the P–S reaction mechanism. Moreover, substrate specificity is essential in determining the biosynthesis pathways of BCAs and monoterpenoid indole alkaloids (MIA). Furthermore, substrate specificity is crucial in biosynthesis engineering of the production of unnatural compounds [60].

STR-like proteins

Several proteins have similar structures with STR in the natural world. Although many members of the STR family have been found in plants and animals, theire relationship have not been fully understood [28, 62]. Rice contains more than 21 STR-like genes; and some of these genes are expressed in all tissues, some of their transcripts are weak in all tissues, and one of them is expressed in a specific tissue alone [63]. *Arabidopsis thaliana* contains four STR-like proteins, where the encoding genes is individually regulated and basal defense may be invovled[64]. STR-like proteins are a large family in both the plant and animal kingdoms, especially human be-

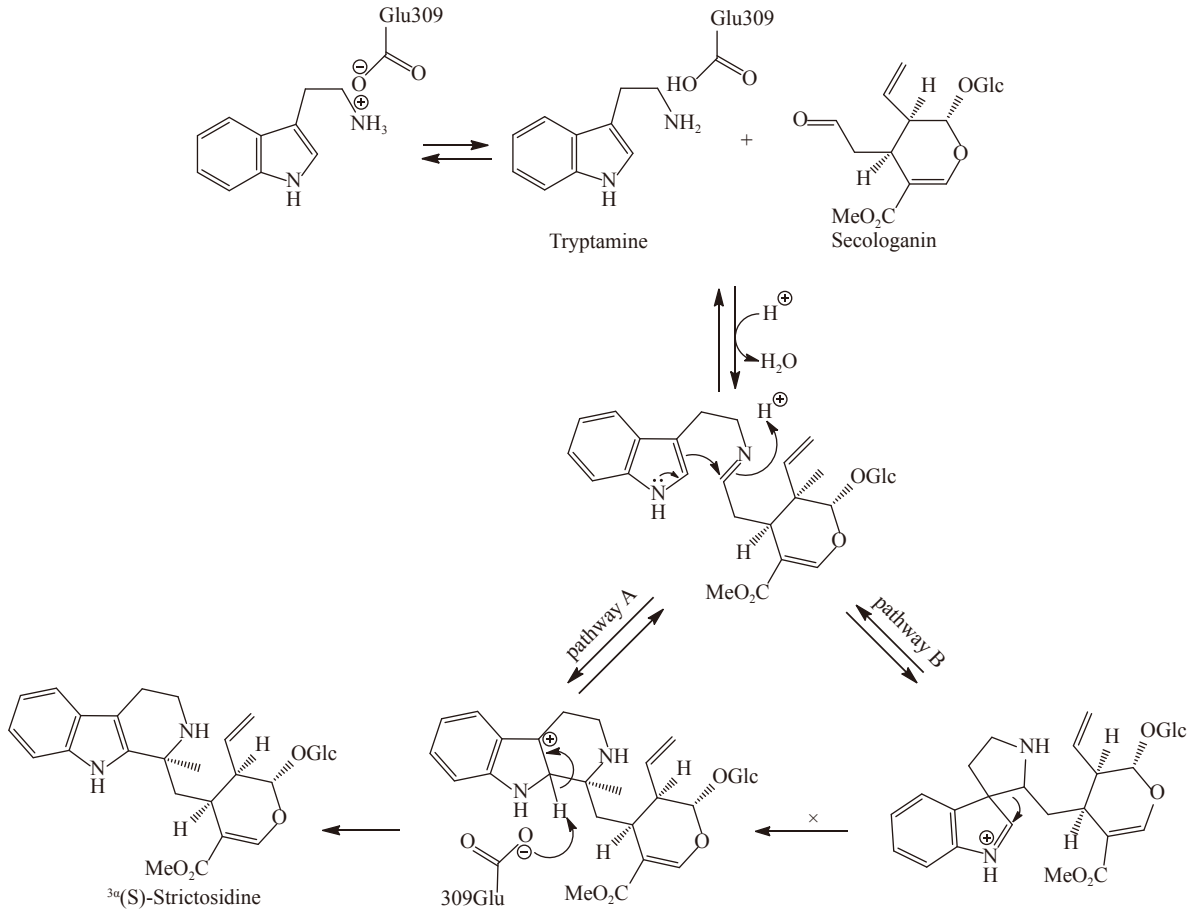


Fig. 7 Mechanism of the STR-catalyzed P-S reaction between tryptamine and secologanin. This mechanism enantioselectively generates the biosynthetic monoterpenoid alkaloid precursor strictosidine [14]

ings. STR-like genes can express in the human brain, kidneys, liver, lungs, pancreas, spleen, and skeletal muscles, and the STR-like family shows the highest expression in the brain [65]. Several proteins, such as diisopropyl fluoro phosphatase [66] from *Loligo vulgaris*, brain tumor NHL domain [67], serum paroxonase [68], and low-density lipoprotein receptor YWTD domain, in the animal kingdom have six-bladed *β*-propeller structures similar to those of STR [69].

STR-like proteins have different functions and may be involved in more than one biosynthesis pathway. Six-bladed *β*-propeller proteins usually function in monomeric states, and few of them are homodimers or oligomers [70]. A summary of six-bladed *β*-propeller proteins is shown in Tables 6 and 7. Such a structure can be found in plants, animals, insects, and bacteria, although the functions of these proteins in different organisms are widely different [68, 69, 71-86]. Proteins in bacteria can act as doorkeepers that recognize foreign matters, whereas proteins in plants and animals can catalyze different reactions without any explicit limit [68, 71-78]. A similar structure provides a similar active center of reaction. Although no coherent conclusion has been proposed about the functional relationship of proteins with such a structure, these catalyzing enzymes usually crack glucoside and acid amides, a reaction that is the opposite of the P–S reaction, among all proteins that contain a six-bladed *β*-propeller structure. Ma *et al*. speculated that these structurally related proteins are evolutionary related, and they may have evolved from a common ancestral *β*-sheet gene [22]. Michael *et al*. argued that these STR-like enzymes function as arylesterase and strictosidine glucosidase that catalyze hydrolytic reactions rather than STR, which catalyzes the P–S reaction [87]. These findings suggest that the STR function may come from an ancestor with a metal-coordinating active site. Such structural features indicate that these proteins may have phylogenetic relationships, although no direct evidence shows similar functions between STR and other *β*-propeller proteins. The similar structures indicate that they may have a similar phylogenetic relationship.

Applications of STR

The compounds derived from strictosidine are shown in Fig. 8. TIAs have effective pharmacological activity; for example, vincristine is widely used for cancer treatment [29]. Vincristine is a valuable but expensive antineoplastic drug because its synthesis is difficult. Therefore, the limited supply of alkaloids must be addressed, and a sustainable way of

Table 2 Reaction between small-molecule aldehydes and tryptamine [56-59] (+, reaction; −, no detected reaction; /, no literature)

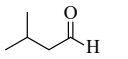
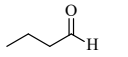
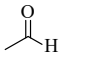
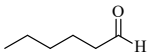
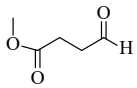
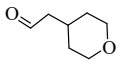
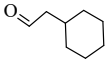
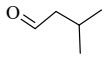
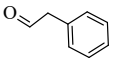
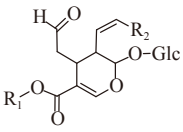
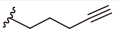
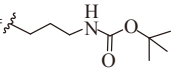
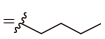
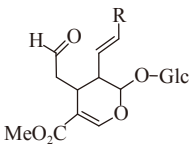
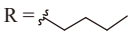
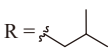
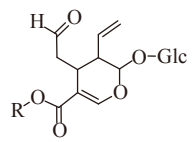
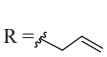
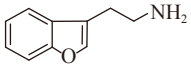
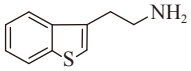
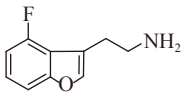
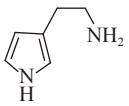
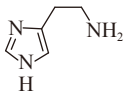
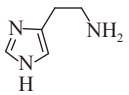
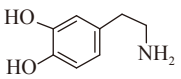
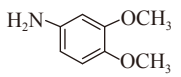
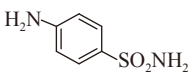
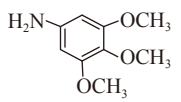
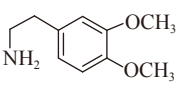
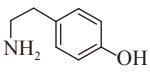
Substrates	CrSTR	RsSTR	OpSTR
	+/−	+	+
	+	+	+
	+/−	+	+
	+/−	+	+
	+	+	+
	+	/	+
	−	/	+
	−	/	+
	−	/	+
	−	/	+
	−	/	+
	−	/	+
	−	/	−
	−	/	−

Table 3 Substrate specificity study for aldehyde substrates (+, reaction; −, no detected reaction) [20, 56, 57]

Substrates			CrSTR
	R1 = 	R2 = H	+
	R1 = 	R2 = H	+
	R1 = 	R2 = H	+
	R1 = 	R2 = 	−
	R1 = 	R2 = 	−
	R = 		−
	R = 		−
	R = 		+
	R = 		+

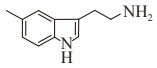
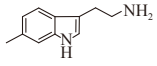
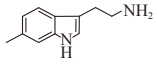
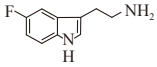
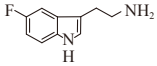
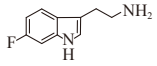
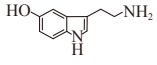
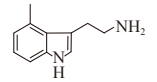
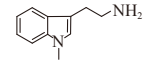
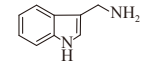
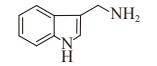
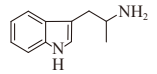
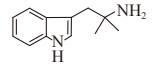
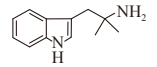
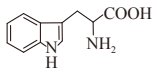
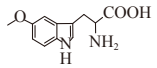
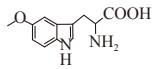
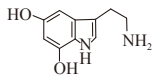
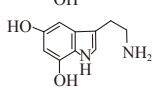
manufacturing such valuable compounds should be developed. As a part of TIAs, BCAs can potentially treat of

Table 4 Substrate specificity studies for amine without indole structure [22, 50, 56, 61] (+, reaction; −, no detected reaction; /, no literature)

Substrates	RsSTR	CrSTR	CpSTR
	/	+	/
	/	+	/
	+	+	/
	+	+	/
	/	−	/
	/	−	/
	−	−	/
	/	+	/
	/	+	/
	/	+	/
	/	+	/
	/	+	/
	/	+	/

many diseases. Ajmalicine has been proved to possess pharmacological effects on cognitive disorders and cerebral and neurosensory impairments by decreasing the loss of biological free energy for phosphorylation that acts at cerebral mitochondrial levels [88, 89]. Some simple BCAs also have efficient pharmacological activities. For instance, harmane, which exhibits anticholinesterase activity [90], is a new potential medicine for Alzheimer's disease. Harmaline and harmine also have similar therapeutic effects [7]. The biosynthetic pathways of TIAs and BCAs clearly show that both come from the same precursor compound strictosidine, which is condensed from tryptamine and secologanin through STR [27, 58]. TIA synthesis in plants is strictly regulated at the level of genes and enzymes, which are differentially expressed due to

Table 5 Reaction between tryptamine derivatives and secologanin for substrate specificity studies [22, 50, 56, 61] (+, reaction; –, no detected reaction; /, no literature)

Substrates	RsSTR	CrSTR	CpSTR
	–	–	–
	–	–	+
	+	+	/
	+	+	/
	+	+	/
	+	+	/
	+	+	–
	/	+	/
	/	+	/
	–	–	/
	/	–	/
	/	–	/
	/	+	/
	/	–	/
	/	/	–
	/	/	–
	/	/	–
	+	/	/
	–	/	/

the influence of several developmental, ecophysiological, and environmental signals [91, 92]. Thus, upregulating STR gene expression is important in obtaining TIAs and BCAs that are difficult to acquire. The factors that influence STR gene expression have been investigated. A thorough insight of the entire biosynthesis pathway of strictosidine helps in understanding plant metabolism. The biosynthesis pathway is affected by numerous factors, including the environment, transcription factors, genes, elicitors, and inhibitors [93, 94]. The factors influencing STR gene expression and its further applications are discussed below.

External environment

The rate of P–S reaction was controlled by the final deprotonation step when the formation of iminium was rapid [51]. The STR gene was upregulated under UV-B irradiation, which substantially increased strictosidine content and TIA accumulation [91]. When *Uncaria tomentosa* roots were cultured, increasing peroxidase activity by eliciting with H_2O_2 upregulated STR activities [38]. The plant growth environment, including light conditions, oxygen content, and water pH, can affect STR expression.

Strictosidine and other related compounds in *C. roseus* plant have been positioned by imaging mass spectrometry and live single-cell mass spectrometry. The results showed that they distributed in epidermal cells or idioblast cells, respectively [95, 96]. So, the transporters of precursor compounds and products may affect the efficiency of STR [97]. Strictosidine content in mechanically wounded leaves increased at 48 and 72 h, a possible result of "nuclear time bomb" mechanism [92]. Salinity and low-temperature stress led to STR regulation [98]. STR activation can be a plant defense strategy [94] and a response to changes in the external environment. STR gene expression markedly increased by eliciting methyl jasmonate and jasmonic acid [38, 99, 100], which are damage-related plant hormones and signaling molecules [101]. The inoculum density of plant cells affected anthranilate synthase and TDC activities, which directly influenced TIA production [102]. Immobilization by adhesion to glass fibers altered STR activity in suspension-cultured cells [103].

TIAs can be remarkably accumulateed as a result of high concentrations of ethylene and copper [93]. Phytohormones downregulated STR gene transcription, which is an indispensable part of cell division and growth [104]. Ag^+ and methyl jasmonate upregulated STR gene expression via transcription factors, such as octadecanoid-responsive *Catharanthus* AP2-domains [105]. Other phytohormones, including 2,4-dichlorophenoxyacetic acid, salicylic acid and abscisic acid, did not enhance TIA accumulation [106]. Real-time PCR revealed that STR gene expression was substantially higher in old leaves than in young leaves where auxin content was higher [107, 108]. In plant cell microenvironment, a dynamic equilibrium adjusts the balance of STR and auxin level. STR gene expression is a vital part of alkaloid yield in plants. Ageez et al. found that the STR gene was not expressed in female *Silene latifolia* but in male ones [109]. STR gene expression in different tissues is obviously different, resulting in distinct TIA profiles [93]. TIA accumulation has no direct relation with chlorophyll formation, which is indispensable to green cells [110]. Thus, various kinds of plant hormones regulate and control STR gene expression.

Expression of the STR gene

The STR gene promoter contains a G-box (CACGTG)

Table 6 Proteins that contain six-bladed *β*-propeller structure (except animals and plants)

Protein	Source	Function	Reference
Serum paraoxonase 1 (PON1)	rePON1-G2E6 expressed in E. coli B834 (DE3) cells	Catalyzes the hydrolysis	[68]
Lectins	*Aleuria aurantia*	Molecular recognition	[71]
Arb93A	*Fusarium graminearum*	Glycoside hydrolases	[72]
Abnx	*Penicillium chrysogenum*	Glycoside hydrolases	[73]
TolB	*E. coli* (carrier: yeast two-hybrid vectors pAS2-1 and pACT2)	Killing bacteria	[74]
Gluconolactonase	XC5397 gene fragment was from X. *campestris* pv. *campestris* str.	Function in the glucose secondary metabolic pathways	[75]
Protein brain tumor (Brat)	*Drosophila*	RNA-binding	[76]
LJM11	*Lutzomyia longipalpis*	Protective immunity	[77]
Drp35	*Escherichia coli* strain B834 (DE3), harboring Drp35 expression vector and pT-RIL	Calcium-dependent lactonase activity	[78]

Table 7 Proteins that contain six-bladed *β*-propeller structure (animals)

Protein	Source	Function	Reference
Diisopropylfluorophosophatase (DFPase)	*Loligo Vulgaris*	Unkown	[79]
Major royal jelly protein 1 (MRJP1)	Apis mellifera	Unknown	[86]
Kelch-related protein 1 (Krp1)	*Rattus norvegicus*	Assembly of myofibrils	[80]
Host cell factor 1 (HCF-1)	Human 293T cells	Transcriptional activation of gene	[81]
Senescence marker protein-30 (SMP-30)	Mouse liver	Regulation of calcium homeostasis and protection of cells from apoptosis	[82]
Carp FEL	frozen carp eggs	One of lectins	[83]
CyRPA	*Plasmodium falciparum*	Inhibition of parasite growth	[84]
PAL	CHO-DG44 cell line	Function as peptidyl-α-hydroxy- glycine α-amidating lyase	[85]
Low-density lipoprotein receptor (LDLR)	CHO cells	Carry cholesterol-containing lipop- rotein particles into cells	[69]

cis-regulatory sequence located around −105. Pasquali *et al*. found that knocking out G-box-binding factor (GBFs) had no significant effect on STR gene expression, but the STR G-box element interacted with nuclear GBFs [111, 112]. More than one type of GBFs is generally present in plants. Siberil et al. isolated GBF cDNA *Crgbf1* clones, expressed these clones in plasmids, and then analyzed the binding specificity of their protein products through competitive electrophoresis mobility and saturation binding assays [113]. CrGBF1 showed high binding specificity to G-box1 (CACGTG) but lower affinity to other G-box-like elements. The STR gene seems to depend on novel regulatory mechanisms because GBFs may influence STR expression, but the G-box is not essential for STR expression.

Furthermore, STR gene upstream sequences directly increase *β*-glucuronidase expression [111]. Chen *et al*. downregulated sly-miR1916 expression, a nonconserved miRNA that responds to various stresses, in *Solanum lycopersicum* to upregulate the STR gene. The miR1916 overexpression decreased the STR gene transcript level and reduced its tolerance to drought in tomato [114]. Such results indicated that the STR gene in different plants has similar expression features. The mitogen-activated protein kinase gene can regulate the STR gene as a response to ethylene in premature leaves.

STR gene expression is influenced by transcription factors, which are proteins that assist transcription initiation. The transcription factor ORCA3 is a major regulator of primary and secondary metabolisms in many plants [115-117]. ORCA3 and SG co-overexpression resulted in marked increases in TIAs. However, overexpressing ORCA3 and controlling the glucocorticoid-inducible promoter did not change the amounts of TIAs [118]. The zinc finger protein family can bind to different fragments of STR promoters and repress STR promoter activities [116, 119]. The soybean transcription factor GmMYBZ2 reduced catharanthine production by upregulating zinc finger proteins and downregulating ORCA3 [120]. AP2/OpERF2 suppression resulted in reduced gene expression in the secologanin–strictosidine pathway, including TDC, loganic acid-O-methyltransferase, secologanin synthase, and STR [121]. Methyl jasmonate and jasmonic acid also act as transcription factors [122, 123]. Endophytes CATDLF5 and CATDLF6 were found to substantially enhance the indole alkaloids content in plant of *C. roseus* without affecting the primary metabolism of the host plant [124]. Gene translation to protein is regulated and controlled by numerous factors, such as base pair sequences and transcription factors, and they simultaneously influence each other.

Other proteins involved in the biosynthesis pathway

Product efficiency is influenced by several genes and proteins due to the complicated biosynthesis system. Upstream reactions of P-S reaction include many other reactions, such as mevalonate pathway and methylerythritol path-

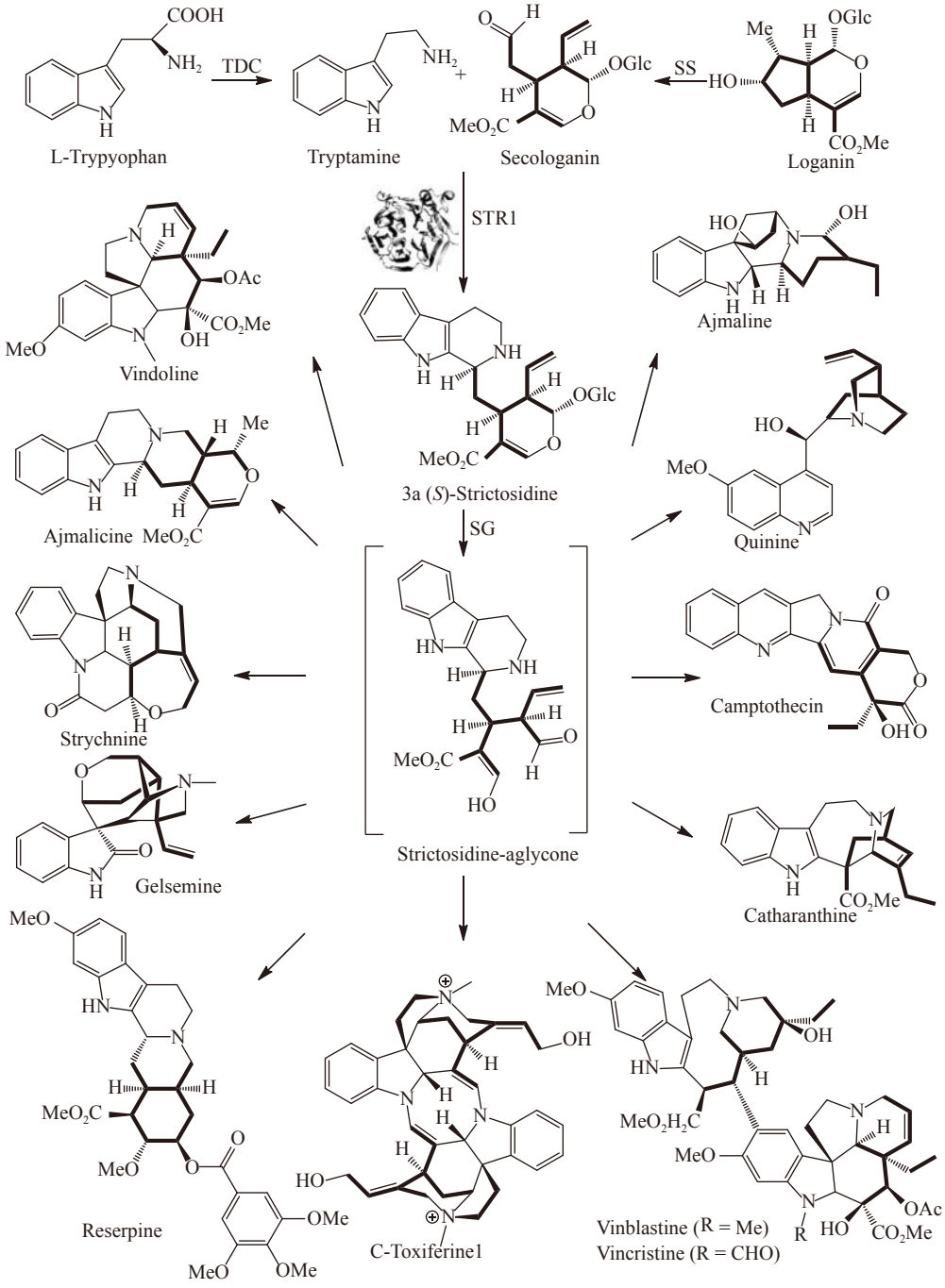


Fig. 8 Strictosidine as the central intermediate in indole alkaloid biosynthesis. STR catalyzes the enantioselective condensation of tryptamine and secologanin, leading to strictosidine as a central reaction in the biosynthesis of the entire family of mono-TIAs in plants [14]

way [125, 126]. To let the article more focused, this part just concentrates on STR and adjacent reactions. The expression of STR has a positive correlation with TIA accumulation [127]. Cui et al. found that TIA yield increased as a result of G-8-H and STR co-overexpression. G-8-H and STR produced a synergistic effect on TIA accumulation [128]. In *rol*-integrated cell suspensions, TDC and STR genes were successfully overexpressed [129]. The amounts of alkaloids were higher in transgenic plants that overexpressed TDC and STR than in normal plants [130], and alkaloids simultaneously enhanced the relative levels of SG and DAT gene transcripts [131]. High TDC activity appears to have adverse effects on plants, and TDC overexpression is not necessary for alkaloid accumulation. High rates of tryptamine synthesis still occurred under low TDC activity conditions [132]. Furthermore, tryptamine accumulation can regulate TDC expression and reduce the flux through feedback inhibition [132, 133]. By contrast, the STR gene appears to be necessary, and an appropriate ratio of

TDC and STR expression can achieve high TIA production [134]. STR and TDC expression levels increased when fortified with synthetic precursors, including secologanin, tryptophan, cyclooxygenase inhibitor (naproxen), acetyltransferase elicitors (acetic anhydride), and hydroxylase elicitors (hydrogen peroxide) [129]. Root cultures with a glutathione biosynthesis inhibitor (buthionine sulfoximine) and hydrogen peroxide also increased STR expression [38]. Iridoid synthase activity toward geraniol and 10-hydroxygeraniol resulted in nonproductive syntheses of citronellol and 10-hydroxycitronellol [135]. *N*-methyltransferase activity is also vital in TIA synthesis [136].

Many other metabolic enzymes are important for strictosidine synthesis. Alcohol dehydrogenase and CYP71D1V1 can control the rearrangement and chemical diversification of strictosidine [39]. CYP72As catalyzed stereoselective hydroxylation and C–C bond cleavage reaction to obtain a ring-opening product with two functional groups, which are essential to secologanin synthesis [137]. The enzyme NADPH cytochrome P450 reductase is vital for the activities of G10H and other P450 monooxygenases [138]. Geissoschizine synthase and ethyl methyl sulfonate controled TIA production in *C. roseus* mutants [139]. Tabersonine 16-hydroxylase and deacetoxyvindoline-4-hydroxylase consecutively took part in the conversion of tryptophan to strictosidine [101].

STR gene copy, transcription, and translation are influenced by base pair sequences, transcription factors, and other proteins. Such influencing elements are commonly used by other gene expressions, and there is no specific one.

Synthesis of STR in other carriers

The yield of target compounds can be increased by upregulating the STR gene or co-overexpressing it with other genes. cDNA clones from the STR of *R. serpentina* expressed in *E. coli* are the first examples of genes involved plant secondary metabolism that have been expressed in an active form in a bacterium [140, 141]. McKnight *et al*. modified cDNAs that encoded the STR of *C. roseus* and successfully knocked in the cDNA to tobacco plants [142]. STR was prepared through the transgenic yeast *Saccharomyces cerevisiae*, and strictosidine was obtained by treating *Lonicera japonica* leaf extracts as secologanin source [143]. Synthesis without a secologanin source in *S. cerevisiae* also succeeded [135]. The biosynthetic pathway was targeted to mitochondria and plasmid-free yeast strain was engineered to product strictosidine [144]. A dual vector for chemoenzymatic synthesis in *E. coli* was developed to increase alkaloid production. In this synthesis, the STR gene was successfully expressed and played an indispensable part [145]. Once all the genes involved in the pathway are clear out, a host organism is more convenient for cultivation than a plant to increase the production of the desired compounds. Biosynthetic engineering techniques are advancing. New technologies can now import exogenous genes to recipient cells and complete gene replication, transcription, and expression. These techniques can also be applied in importing the STR gene to induce the recipient cell to automatically generate strictosidine. Brown et al. added several genes, including 11 genes involved the strictosidine biosynthetic pathway and three genes that enhanced P450 activity, to yeast [18]. They replaced the ERG20 gene and imported the AgGPPS2 and mFPS144 genes from *Abies grandis* and *Gallus gallus* to synthesize geranyl pyrophosphate, which is the precursor of geraniol [146, 147]. The production and downstream pathway intermediates of strictosidine can be observed in the final production *via* LC-MS analysis, and the G-8-H gene plays an important role in strictosidine synthesis [148]. BCA and TIA biosyntheses can be based on the results of strictosidine-engineered technology. This technology is a valuable method for building a microbial system for the production of unavailable and complicated compounds. Introductory synthase strategies and methods based on STR can produce numerous novel alkaloids with great scaffold diversity by combining chemical and enzymatic implementations [148]. 12-Aza-strictosidine was synthesized successfully via a chemoenzymatic method [149]. STR was successfully expressed in insect cell cultures using a baculovirus-based expression system [150].

Conclusions

With our increased understanding of biosynthetic pathways and the advances of novel techniques, great progress has been achieved on STR studies, which allows further researches of enzymes. However, numerous problems remain to be addressed. First, although the structure of STR is determined owing to the development of structural biology research, the relationships among other six-bladed *β*-propeller proteins remain unclear. Their structures are similar, but currently no direct evidence supports their connections regarding function. These proteins may be evolved from the same ancestor. Future investigation must be carried out to analyze the structures and functions of these proteins. Second, biosynthetic engineering of strictosidine is nascent. The genes and proteins involved in biosynthesis pathways remain to be identified. Studies on the factors influencing STR gene expression are not comprehensive. Future researches can focus on specific genes and proteins, and the entry point can be the difference in the gene sequences of different plants. These differences may explain why the same substrate catalyzed by STR from different plant species lead to different results. Finally, BCAs can be derived from other compounds other than strictosidine and the reaction catalyzed by norcoclaurine synthase and McbB. Such catalytic reactions may produce interactive effects to a certain extent because they use similar substrates and products. In summary, considerable progress has been achieved on SRT studies, but future efforts are strongly needed to explore its origin and influencing factors.

Abbreviations

AChE: Acetylcholin esterase; BCAs: *β*-Carboline alkaloids; CpSTR: STR comes from *Cinchona pubescens*; CrSTR:

STR comes from *Catharanthus roseus*; G-8-H: Geraniol-8-hydroxylase; MIA: Monoterpenoid indole alkaloids; OpSTR: STR comes from *Ophiorrhiza pumila*; ORCA: octadecanoid-responsive Catharanthus AP2-domain; P-S reaction: Pictet-Spengler reaction; RsSTR: STR comes from *Rauvolfia serpentine*; SG: Strictosidine glusidase; SS: Secologanin synthase; STR: Strictosidine synthase; TDC: Tryptophan decarboxylase; TIAs: Terpenoid indole alkaloids.

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