Biosynthesis and metabolism of β-D-salicin: A novel molecule that exerts biological function in humans and plants

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A B S T R A C T

β-D-Salicin **1** (Mahdi et al. [8]) is an interesting medicinal phytochemical that exhibits cross functions in plants and humans immunologically. This molecule **1** (Mahdi et al. [8]) has attracted the attention of scientists in various interdisciplinary fields, including chemistry, pharmacology and medicine. The biological cross functions of β-D-salicin **1** (Mahdi et al. [8]) serve in plant survival and healing processes *via* salicylic acid **2** (Pierpont [23]). Thus, this raise a question whether plant biosynthesis and human metabolism crosstalk to induce therapy *via* molecular recognition. If so, biotechnology and bioinformatics are significant techniques for new strategies in drug development. Thus, understanding the biosynthesis, metabolism and the cross-molecular setting of recognition may encourage further discussion and research on its medicinal and biological activity virtues.

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1. Introduction

The willow tree, like any other medicinal plant species, can be considered as a bioreactor for the biosynthesis of many phytochemicals, including β-D-salicin **1**. These phytochemicals are recognised as secondary metabolites, which contribute to the biology of plants, as they are essential for growth, reproduction and have important roles in the ecological survival of plants against biotic and abiotic stress [1–7]. The accumulation of knowledge on phytochemistry, pharmacology and pharmacognosy and the rapid development of analytical techniques in chemistry all have profoundly contributed to the discovery of β-D-salicin **1** and its metabolite, salicylic acid **2**. The elucidation of the chemical structures of these two phytochemicals, **1** and **2**, leads the discovery of the most common anti-inflammatory drug, acetylsalicylic acid **3** or aspirin [8]. In this respect, researches have recognised the essential steps for exploiting plants in drug discovery and development. These steps include the identification of natural products, characterisation of the chemical structures of the bioactive molecules, investigating their pharmacological potentials and identifying the target active sites. The ethnomedical usage of plants and the retrospective pharmacological activities have also contributed to the identification of biologically active phytochemicals [9–10]. Per se, β-D-salicin **1** and salicylic acid **2**

from willow have been identified to exert vital pharmacological roles in modulating the inflammatory process and inhibition of the activation of NF-κB, and subsequent down regulating COX-2 expression [11–12]. In addition, our recent results using Schrödinger software showed that β-D-salicin **1** has better binding affinity with COX-2 (docking score = −9.966) and NK-κB (docking score = −9.274) compared to acetylsalicylic acid **3** (docking score for COX-2 = −5.412; for NK-κB = −5.525) [13]. Furthermore, salicylates exhibit other biological activities, including anticancer and anti-proliferation [12]. The significance of β-D-salicin **1** molecule may encourage further understanding into its cross-biological function. Therefore, the aim of this article is to explore the mechanistic biosynthetic pathways of β-D-salicin **1**, its metabolism and discuss the genetic cross-talk between pants and humans.

2. Phytochemistry of β-D-Salicin 1

β-D-Salicin **1** or 2-(hydroxymethyl) phenyl-*O*-β-D-glucopyranoside is the first phenolic glycoside discovered in nature with a molecular mass of 286.27782 g/mol. Its IUPAC name is (2R,3S,4S,5R,6S)-2-(hydroxymethyl)-6-[2-(hydroxymethyl) phenoxy]oxane-3,4,5-triol. β-D-Glucose **4** moiety of β-D-salicin **1** contributes to all five chiral carbon centres. The chemical structure of β-D-salicin **1** encompasses β-D-glucose **4** and 2-hydroxybenzyl alcohol, or salicyl alcohol **5**. β-D-Salicin **1** contains seven oxygen atoms, as H-bond acceptor and five hydroxyl groups, as H-bond donors. It **1** also possesses nine rotational bonds that

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control its conformational structure. In addition, the β-D-glucose **4** and salicyl alcohol **5** moieties are bonded by β-1,1′-D-glycosidic bond. These chemical features contribute to the polarity of β-D-salicin **1**. Therefore, the extraction of β-D-salicin **1** requires a polar solvent system, such as boiling water and ethyl alcohol. In addition, the presence of D-glucose **4** moiety contributes to the enhancement of physcochemical properties of β-D-salicin **1**.

3. Biosynthesis of β-D-Salicin 1

Although there have been long-standing biotic and abiotic interests in β-D-salicin **1**, no defined biosynthetic pathway, genes or enzymes have been illustrated in the literature [14–15]. Nonetheless, adapting the biotechnological approach and utilising leave tissues and radio labelled precursors have elucidated some biosynthetic aspects of β-D-salicin **1** in *Salix* and *Populous* [16–17]. It reveals that the biosynthesis of β-D-salicin **1** is associated with phenylpropanoid pathway that starts with L-phenylalanine **6** (Scheme 1). Using radiolabled precursors indicate that the biosynthesis of β-D-salicin **1** encompasses five steps: deamination, ortho-hydroxylation, β-oxidation, C2 unite elimination and glucosylation [7,16].

4. Formation of *trans* cinnamic acid 7

L-Phenylalanine **6** is available in plants and readily biotransforms into *trans* cinnamic acid **7** by phenylalanine ammonialyase (PAL) [18]. Thereby, plants produce a large number of organic compounds *via* this biotransformation [19]. The catalysis of L-phenylalanine **6** involves deamination of the amino group of α-amino acid. The mechanism of this biotransformation involves the formation of an enzyme-substrate complex, generating a carbonium ion intermediate which subsequently induces the elimination of the 3-*pro-S* proton giving *trans*-cinnamic acid **7** in a stereospecific manner (Scheme 2).

5. C2 unite elimination

Salix and *Populous* utilise *trans*-cinnamic acid **7** and *trans*-coumaric acid **8** in the synthesis of essentially benzoyl-SCoA **9** and salicyloyl-SCoA **10**, respectively (Scheme 2). The incorporation of radiolabelled **7** or **8** in *Salix* or *Populous* leave tissues can readily be transformed stereospecifically to 3-hydroxy-3-phenyl-propanoic acid **11** or 3-hydroxy-3-(2-hydroxyphenyl)-propanoic acid **12** *via* CoA-dependent β-oxidation [7,20]. Subsequently, 3-hydroxy propanoate side chain of compounds, **13** or **14**, undergo C2 unit elimination to yield **9** or **10** *via* retro Claisen

condensation (Scheme 2). The mechanism of biotransformation, in the last two steps, is analogous to the metabolism of fatty acids in humans [16,20–21]. The elimination of the C2 unit involves the formation of β-oxophenyl propionyl-CoA **13** or β-oxo-orthohydroxyphenyl propionyl-CoA **14** which is followed by the nucleophilic attack by thiolase at β-carbonyl group, forming an enzyme-substrate complex **15** or **16**, respectively. These two complexes, **15** and **16**, subsequently, undergoes α-β-C–C cleavage, resulting in the formation of the following intermediates: **17**, **18**, **19**. Protonation of **17** gives acetyl CoA **20** while the intermediate **18** and **19** undergo nucleophilic attack by acetyl S-CoA to release the enzyme and form benzoyl-SCoA **9** and salicyloyl-SCoA **10**, respectively (Scheme 2).

Plants modulate the phenylpropanoide pathways by interconverting benzoate secondary metabolites in response to the plant's physiological requirement. Therefore, the exact mechanism of β-D-salicin **1** biosynthesis may seem difficult to justify. Using *Salix* and *Populous* leaf tissue indicated that the downstream of β-D-salicin **1** biosynthesis involves inter conversion of different simple phenolic molecules, including benzaldehyde **21**, benzoic acid **22** and benzoyl-SCoA **9** compounds in plants [7,16,22,23]. The biotic transformation of cinnamic acid **7**, for example, can undergo direct *ortho* hydroxylation to give 2-hydroxycinnamic acid **8** or C2 elimination to give benzaldehyde **21** (Scheme 3). Benzaldehyde **21** can also be hydroxylated at *ortho* position to give 2-hydroxybenzaldehyde **23**. Feeding the leave tissue of *S. purpurea* with radiolabelled benzoic acid **22** or benzyl alcohol **24** gave benzaldehyde **21** *via* reduction or oxidation reaction, respectively [7,16]. Further biotic transformation of compounds **22** and **24** gave salicyl alcohol **5**, the precursor of β-D-salicin **1** (Scheme 3). In addition, benzoyl-SCoA **9** undergoes a reduction reaction to give benzyl alcohol **24** or benzoic acid **22** (Scheme 3).

In addition, there are other benzoate secondary metabolites that have been found in *Populous*, which contribute to the biosynthesis of phenolic glycosides. These benzoates are 1-hydroxy-6-oxo-2-cyclohexene-1-carboxylic acid **26**, benzyl 6-hydroxy-2-cyclohexen-on-oyl **27** and salicyl 6-hydroxy-2-cyclohexen-on-oyl **28** [7,22,23]. The final step, in the biosynthesis of **1**, involves glucosylation of salicyl alcohol **5** at the phenyl hydroxyl group.

6. Glucosylation and its stereospecificity

In *S. populous* and other plants, salicylaldehyde **23**, its counterpart SCoA active form **9** or salicyl alcohol **5** can undergo glycosylation with D-glucose **29** [7,16,24–26]. Scheme 4 shows the
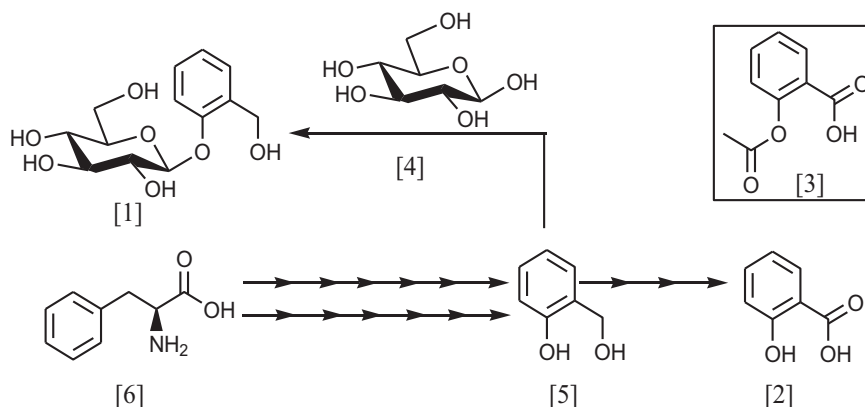
**Scheme 1.** An overview of the β-D-salicin **1** biosynthesis.
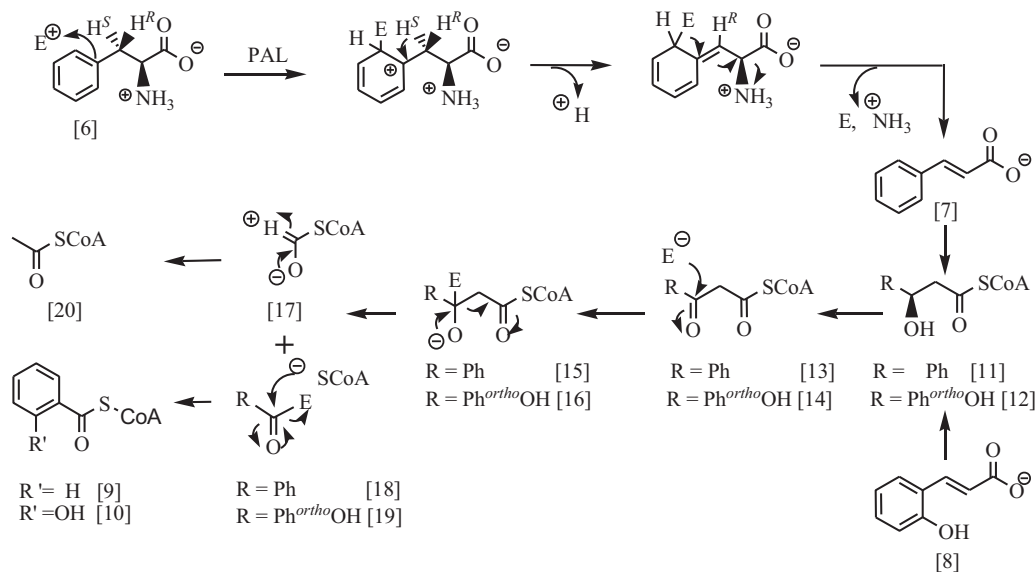
Scheme 2. The stereospecific biosynthetic transformation of ʟ-phenylalanine for generating benzoic acid-related analogues.

direct and indirect routes that involve the formation of β-ᴅ-salicin **1**. Radiolabelled salicylaldehyde **23** was readily glucosyled to yield β-ᴅ-helacin **30** when fed to *S. purpurea* which, subsequently underwent reduction at the carbonyl group to give β-ᴅ-salicin **1** [7,16]. In addition, using radiolabelled β-ᴅ-helacin **30** undergoes similar reduction to give β-ᴅ-salicin **1** [27]. Research also found that using radiolabelled salicyl alcohol **5** can be directly incorporated in the synthesis of **1** (Scheme 4) [16]. However, literature indicated that salicyl alcohol **5** is not the direct precursor of β-ᴅ-salicin **1** in higher plants. Although salicyl alcohol **5** can undergo glycosylation reaction, it only underwent 46.4% incorporation into β-ᴅ-salicin **1** while 53.6% of it **22** formed *ortho*-hydroxybenzylglucoside **31** [16]. Chemically, there are two types of hydroxyl group that are present in salicyl alcohol **5**: primary and phenolic. In physiological environments, these two

hydroxyl groups are different in their chemical properties. Primary hydroxyl ($pK_a = \sim16$–19) is amphoteric, while phenolic hydroxyl tend to be acidic ($pK_a = \sim8$–10). These chemical properties may play an essential role in the selectivity of which type of hydroxyl group preferably undergoes glucosylation. Nonetheless, with a single enzyme, the ratio of glucosylation is controlled by the stereo-specificity or by the relative biochemical reactivity of hydroxyl groups.

The stereochemistry of the β-glycosidic bond formation in β-ᴅ-salicin **1** is based on transglycosylation of glycan (ᴅ-glucose) with an aglycan (benzoate) compound. The mechanism that controls the configuration of the β-bond requires two carboxylate residues on the enzyme that are spatially proximal within about 6.0 Å [28]. In this mechanism, the two nucleophilic carboxgylates participate in the transglucosylation, as illustrated in Scheme 5.
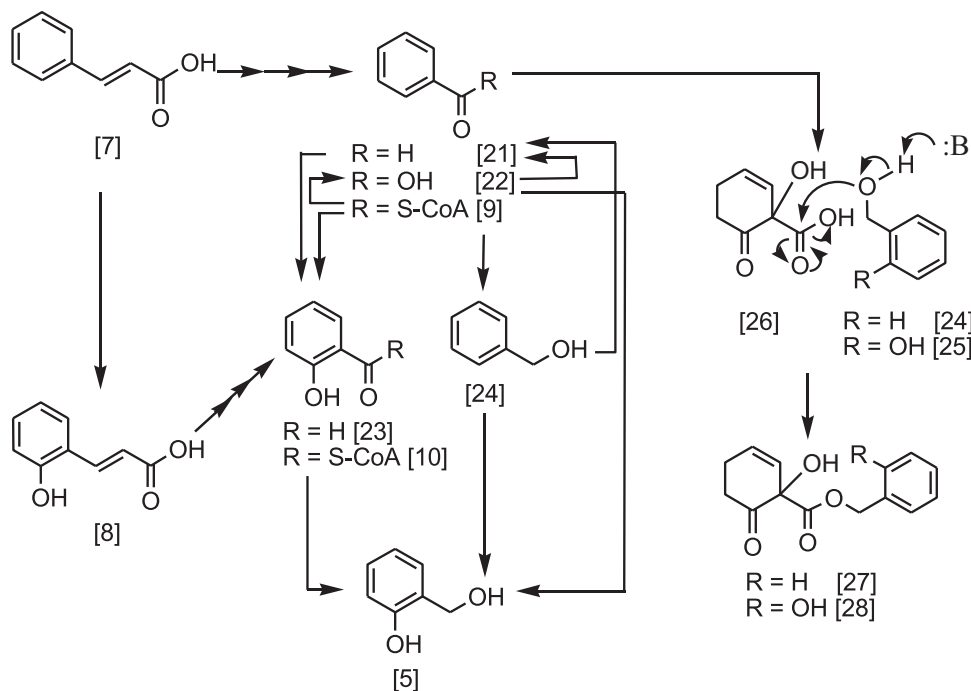
Scheme 3. Incorporation of radiolabelled compounds in the biosynthesis of β-ᴅ-salicin **1**.
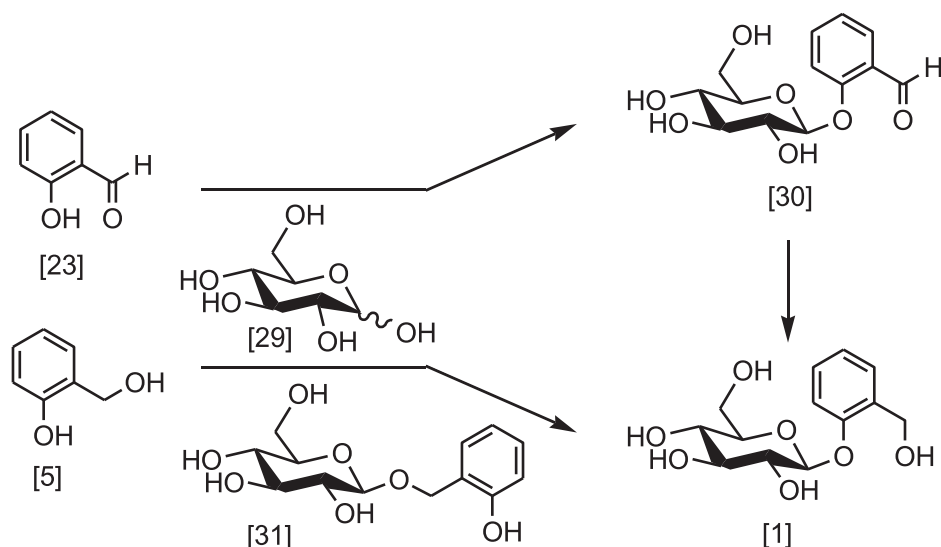
Scheme 4. Glucosylation of salicyldehyde **23** and salicyl alcohol **5**.

The nucleophilic carboxylate of glucosidase attacks the anomeric centre of D-glucose **4** to form an enzyme-substrate complex, while the acid/base residue protonates the glycosidic oxygen and subsequently activates a compound acceptor to form the transglycosylated product **1** [28].

7. Metabolism of β-D-Salicin 1

β-D-Salicin **1** is a pro-antiinflammatory drug which upon oral administration, is metabolised into the pharmacological active form, salicylic acid **2**. This metabolic step takes place in the gastrointestinal tract and blood stream which involves glycon hydrolysis and oxidation of benzyl carbon. Similarly, acetylsalicylic acid **3** is also hydrolysed into salicylic acid **2** and acetic acid.

The route to the metabolism of these drugs has been associated with esterases that are found in the intestinal mucosa and serum cytosol [29]. Salicylic acid **2** undergoes further metabolism in the liver and kidney, as part of drug clearance (Scheme 6). Cytochrome p-450 enzyme system in mitochondria is capable of catalysing salicylate into different metabolites, including 2,3,5-trihydroxy benzoic acid **32**, 2,5-dihydroxy benzoic **33** acid and catechol **34**; the decarboxylated analogue of compound **35** [30–32]. The biotransformation is likely to involve enzymatic hydroxylation or radical oxidation. In addition, salicylic acid **2** is readily available for conjugation reaction with glycine **36** to form salicyluric acid **37** or D-glucuronic acid **38** to form salicylacyl glucuronide **39** or 1-salicylate glucuronide **40** *via* the formation of ether or ester bonds (Scheme 6).

The mechanism of aryl hydroxylation involves a cyclohexa-dienyl radical intermediate followed by hydroxy radical attack (Scheme 7A). While the biotransformation of **2** compound, in the presence of glycine **36** or D-glucuronic acid **38**, in the liver and kidney gives, respectively, **37** and **39** and **40** compounds. The mechanism of these reactions involves nucleophilic addition of amino group or hydroxyl group to the carbonyl group of salicylic acid **2**, as illustrated in Scheme 7B. In addition, the carboxylic group ($pK_a = 4.5$) is characterised as a week acid, and it is readily available to interact with any congruent amino group ($pK_a = 10.5$) or hydroxyl group ($pK_a = 10$) forming amide or ester bonds, respectively (Scheme 7B).

The interactions of carboxylic group in salicylic acid **2** with macromolecules must be tightly associated with the cellular recognition which mainly depends on the compatibility of the inter-relationship between compounds to interact with each other. Salicylic acid **2** possesses three functional groups, as indicated above, which allow different biochemical interactions to take place within the cellular molecular biology. The functional groups also allow appropriate modifications, with the aim to improve its anti-inflammatory and pro-apoptotic efficacy.

8. Molecular recognition and cross-function of β-D-Salicin 1

The molecular recognition is a fundamental concept of how molecules communicate with their partners in micro-environments. The tool for recognition mainly involves molecular interactions, whereas, functional groups in molecules are the main sources for molecules to interact with each other. The
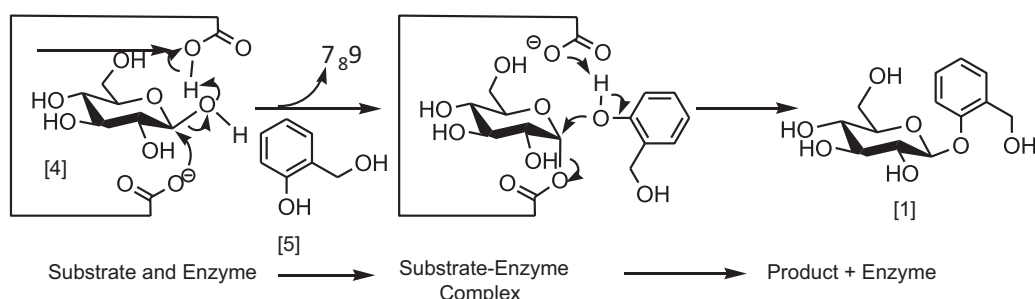

Scheme 5. The mechanism of enzymatic glucosylation of slicyl aalcohol **5**.
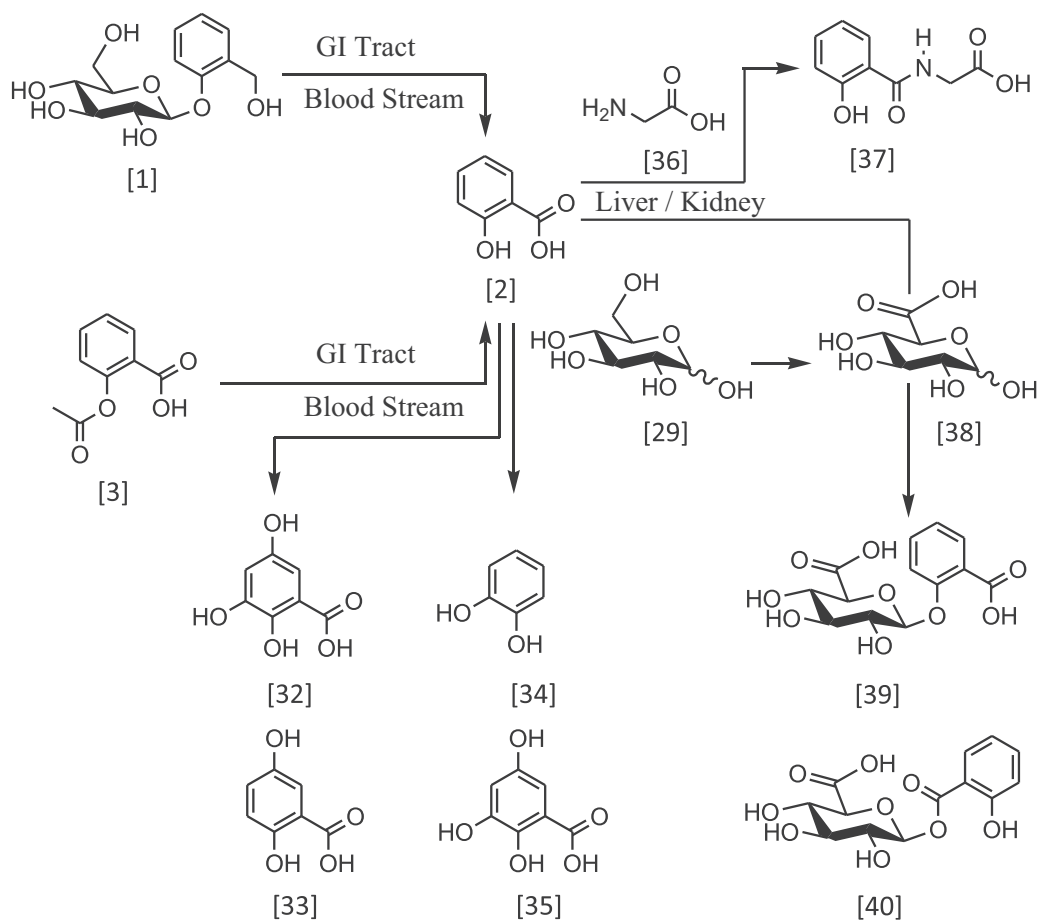
Scheme 6. An overview of the metabolism of β-ᴅ-salicin **1** and related compounds.

simplest example is water (H—O—H) whereas both hydrogen and oxygen atoms contribute to the formation of hydrogen bonding with other water molecules. In addition, molecules containing functional groups (*e.g.* —OH, —SH, —NH) also may interact with each other. β-ᴅ-Salicin **1** and salicylic acid **2** contain mainly hydroxyl groups (—OH) that can interact with cyclooxygenase-2,

causing the inhibition of this enzyme and subsequently down-regulating inflammation [11–12]. Thus, molecular recognition is often the main dynamic process of any signalling cascade. As such, the molecular recognition is vital for understanding drug-receptor interaction, and drug development. In order for the molecule to achieve suitable interaction, the molecular geometry and shape of
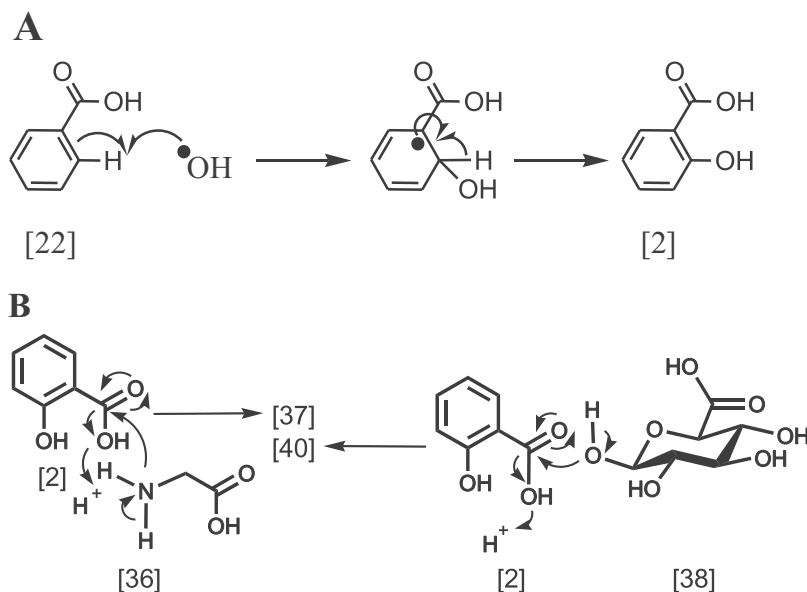
Scheme 7. The mechanism of free radical hydroxylation and glycosidation.

the interacted molecules must mach. Thus, for salicylic acid **2** to be recognised by the active site of cyclooxygenase-2, both must have matched geometry that allows the interaction. However, it is not yet clear how salicylic acid **2** is directly recognised by some inflammatory mediators while β-D-salicin **1** must be metabolised to exert its anti-inflammatory potential. Owing to the random nature of macromolecules to recognise xenobiotic molecules, they may generate an expression on how molecules communicate with each other to produce specific function. However, random interaction may not be suitable in a complex dynamic biological system. It seems most likely that a genetic match occurs between specific phyto-biosynthesis and therapeutic activities to restore inflammatory problems clinically. Per se, humans have identified the diversity of herbal medication according to the type of plant. The earliest explanation to the therapeutic potential of plants goes back to the Doctorine of Signatures, a philosophy that rationalizes the similarity in colour or shape between matched parts of plant and human bodies to coordinate treating an ailment. The other explanation is related to the co-evolution that is associated with close proximity between plant and human. In both point of views, the cross-talk may exist in engineering DNAs in plant and human in a way to complement each other.

Although the structure of DNA, in all living things is a complicated structure, it simply encompasses of only four repeating nucleotide units; adenine, cytosine, guanine and thymine, or respectively ACGT. Therefore, plant and human DNAs are structurally identical in their monomeric composition, but different in the sequence patterns of these monomers, the nucleotides. In order to understand the relationship between biosynthesis and pharmacological properties of specific phytomolecule, it is important to consider the pattern of the encoded enzymes in biosynthetic and pharmacological pathways. The interaction of phyto-molecule with an enzyme requires recognition of amino acid consensus motifs of this enzyme. In addition, the pattern of recognition must have its root in the encoded gene(s) that control both biosynthesis and pharmacology pathways. In this respect, the availability of high-throughput technologies in genome and various databases is considered vital for bioinformatics approach for the analysis of DNA sequence bioinformatically. The genetic approach that encompasses encoded specific gene and or the corresponding expressed proteins may help us to understand the complementary functional relationship of phyto-secondary metabolites. This may encourage the development of new biotechnological strategies for therapeutic intervention of certain clinical cases. Mapping of encoded-related genes and analysis of the nucleotides/amino acids sequences of cascade networks bioinformatically may also facilitate a quick understanding into the pattern of the cross-talk between biosynthesis of a phytomolecule and its pharmacological potential. High-throughput [33], serial analysis of gene expression [34] and massively parallel signature sequencing [35] provide good opportunities for studying sequences and expressions.

9. Conclusion

β-D-Salicin **1** and salicylic acid **2** are interesting phytochemicals that exert cross-biological functions in plants and humans. This cross-function may be linked to the homological nature of DNAs in both plants and humans and can be extended to animals and insects. In this respect, both cell regulatory proteins and nucleic acids, for example, possess the same amino acids or nucleotides repeating units, respectively. The match-up between a phytochemical and the corresponding receptor depend on the molecular recognitions and the stereo-compatibility of the interacted molecules. Therefore, mapping and analysing gene and expressed protein sequences of certain biosynthetic/pharmacologyical related pathways of certain phytochemical bioinformatically may contribute to devising new strategies in drug production. As such, β-D-salicin **1** and salicylic acid **2** may represent good examples in this respect, as both molecules exert biological activities in plants and humans to antagonise cell molecular dysfunction.

Conflict of interests

Author declare that there is no any conflict of interests.

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