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19

Phenylethanoid Glycosides

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ABSTRACT

Phenylethanoid glycosides (PhGs) are plant natural products with multiple bioactivities including antioxidant, antibacterial, antitumor, antiviral, antiinflammatory, neuroprotective, hepatoprotective, immunomodulatory and tyrosinase inhibitory actions. The core structure of PhGs is characterized as hydroxyphenylethyl moiety attached to a β-glucopyranose through glycosidic linkage. PhG isolation / purification, physico-chemical properties, chemical structural characterization, structure-activity relationships, biosynthesis in plants, as well as their functional mechanisms and prominent therapeutic values, has been reviewed in this chapter.

***Key words*:** Phenylethanoid glycosides, Plant natural products, Antioxidants, Antibacterials, Antitumor, Antiviral, Anti-inflammatory, Biosynthesis

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ABBREVIATIONS

Alzheimer's disease, AD; aldose reductase, AR; capillary electrophoresis, CE; correlation spectroscopy, COSY; caffeoyl phenylethanoid glycosides, CPGs; capillary zone electrophoresis, CZE; 1,1-diphenyl-2-picryl hydrazyl, DPPH; electrospray ionization, ESI; ferric reducing antioxidant power, FRAP; hepatitis B virus, HBV; human immunodeficiency virus, HIV; heteronuclear multiple bond correlation spectroscopy, HMBC; high performance liquid chromatography, HPLC; high-speed counter-current chromatography, HSCCC: high resolution HR; heteronuclear single quantum correlation spectroscopy, HSQC; isocampenoside II, ICD; interferon, IFN; liquid chromatography, LC; lipopolysaccharide, LPS; microemulsion electrokinetic chromatography, MEKC; minimum inhibiting concentration, MIC; mass spectrometry, MS; tandem mass spectrometry, MS/MS; macroporous resin, MR; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, MTT; normal human keratinocytes, NHK; nuclear magnetic resonance, NMR; nuclear Overhauser effect spectroscopy, NOESY; neurotrophic factors, NTFs; Parkinson's disease, PD; photodiode array, PDA; phenylethanoid glycosides, PhGs; phorbol myristate acetate, PMA; phenyl propanoid glycosides, PPGs; quadrapole time-of-flight, QTOF; recombinant human, rh; reactive oxygen species, ROS; tumor necrosis factor, TNF; total phenyl glycosides, TPG; 2,3,5-triphenyl-2H-tetrazolium chloride, TPTZ; thin layer chromatography, TLC; ultra-high performance liquid chromatography, UPLC; ultraviolet, UV.

INTRODUCTION

Phenylethanoid glycosides (PhGs), also known as phenylpropanoid glycosides (PPGs), are natural products of plant origin. Hundreds of PhGs have been isolated, identified, and extensively studied, *e.g*., echinacoside from *Echinacea angustifolia* DC. (*Asteraceae*) and verbascoside (acteoside) from *Verbascum sinuatum* L. (*Scrophulariaceae*) and *Syringa vulgaris* L. (*Oleaceae*) (Fig. 1). Pharmaceutical research has revealed that these compounds have a broad range of bioactivities, including, antioxidant, antibacterial, antiviral, antiprotozoa, antiinflammatory, anti-HBV, antiproliferation, antitumor, neuroprotective, hepatoprotective, immunomodulatory and tyrosinase inhibitory actions, which make PhGs potential clinically relevant in the treatment of heart and liver disease, Parkinson's disease (PD), human immunodeficiency virus (HIV) and cancer. The core structure of PhGs is characterized by a hydroxyphenylethyl moiety attached to a β-glucopyranose through glycosidic linkage. The variation of hydroxyphenylethyl and the addition of saccharide units can result in many complex PhG metabolites. The biogenesis of these compounds comes from the phenylpropanoid metabolic pathway.

This chapter elucidates PhG identification as well as PhG therapeutic utility. Progresses on PhG isolation, purification, analytical approaches, physico-chemical properties, structure and activity relationship, and their biosynthesis and chemical synthesis will be reviewed.

PLANT SOURCE

PhGs are widely distributed in the plant kingdom and have been isolated from families *Scrophulariaceae, Oleaceae, Acanthaceae, Lamiaceae, Verbena, Asteraceae, Plantaginaceae, Orobanchaceae, Magnoliaceae*, most of the species of these families are medicinal herbs, *e.g. Rehmannia glutinosa* (Gaerln.) *Scrophularia ningpoensis,* Hemsl *Cistanche deserticola, Forsythia suspens* (thumb), *Vahl Buddleja lindleyana* fortune*, Plantago asiatica, Teucrium chamaedris, etc.* The accumulation of PhGs is not specific to any plant organs and tissues. PhGs have been isolated from plants roots, bark, leaves, stems, flowers, fruits, aerial parts as well as from callus tissue, suspension cell cultures, adventitious and hairy roots cultures[1–3].

Several hundred of PhGs and their plant origins have been enumerated in previous reviews[1,4]. Many new compounds are still being discovered and investigated. For instance, chionoside, a phenylethanoid triglycoside has recently been obtained from *Veronica beccabunga* L[5], acanmontanoside, a phenylethanoid diglycoside from *Acanthus montanus* (Nees)[6], cistansinenside B from *Cistanche sinensis* (Orobanchaceae)[7], rashomoside A from *Meehania urticifolia* (Miq) Makins[8], four PhGs saccharumosides A-D from sugar maple (*Acer saccharum* MArshall)[9], isoforsythiaside from *Forsythia suspense* (Thunb.)[10], Lianqiaoxinoside B, a novel caffeoyl phenylethanoid glycoside (CPG) from *Forsythia suspense* (Thumb.)[11].

In plants, PhGs usually co-exist with sugars, sugar alcohols, iridoid glycosides, and lignan glycosides and act as the bioactive ingredients in traditional medicines used for treatment of a wide range of human disease such as inter alia bronchitis, tuberculosis, asthma, and inflammation[9,12–19]. Due to the common presence of PhGs in plants, the profiles of PhGs have been used as chemotaxonomic markers to differentiate species and the PhGs chemical profiles combined with other metabolites have been employed to assess the phylogenetic relationships in groups of species[20–25].

For example, PhGs are the major secondary metabolites in *Cistanche* herba including *C. deserticola*, *C. tubulosa* (Schenk), *C. phelypaea*, *C. salsa*. and *C. sinensis* Bock. Acteoside, echinacoside, and 2′ -acety-lacteoside are common components in these species and regarded as chemical markers for identification of *Cistanche* species. Echinacoside and acteoside are the major components in *C. deserticola, C. tubulosa,* and *C. salsa* but only a little in *C. sinensis* in the high performance liquid chromatography (HPLC) profile. Besides, an unusual rhamnosyl moiety on C-6 of inner glucose, 6′ -*O*-rhamnosyl moiety, of PhGs in *Cistanche* species was present in cistansinenside B, poliumoside, and 2′ -*O*-acetylpoliumoside, three novel PhGs in *C. sinensis*, which could also serve as chemotaxonomic markers to differentiate *C. sinensis* from other species[7,26].

Another example is the discrimination and the assessment of phylogenetic relationship of four species in sun hebes using the chemical profiles of iridoid glucosides and CPGs[25]. Although each of the studied species has a characteristic chemical profile the most distinctive chemical character of the group is the universal presence of esters of cinnamic acid derivatives with iridoid and PhGs, especially the unusually high concentration of verminoside, confirming the close relationships in the group and its distinctiveness within New Zealand *Veronica*. In contrast, heliosepaloside and verproside are found in all but *V. lavaudiana,* thus discriminating it from other species. *V. raoulii* and *V. pentasepala* are similar, both containing mussaenoside, verbascoside and one of its derivatives. *V. raoulii* contains relatively small amounts of CPGs. Besides, the presence of mussaenoside and the relatively simple flavonoid profiles, earlier metabolites in the pathway in sun hebes, imply the early origin of the group in the establishment of *Veronica* in New Zealand[25].

STRUCTURE CHARACTERISTICS AND PHYSICO-CHEMICAL PROPERTIES

Chemical Structure Characteristics

The core structure of PhGs is characterized by a hydroxyphenylethyl moiety linked with a β-glucopyranose through glycosidic bond. In most cases the basic structure of PhGs contains a cinnamic acid and hydroxyphenylethyl moiety with a β-glucopyranose through ester and glycosidic bond, respectively. Variation of PhGs occurs in both the sugar and hydroxyphenylethyl moieties. Sugars (such as rhamnose, xylose, apiose, arabinose, galactose and allose) and/or aromatic acids including cinnamic acid, coumaric acid, caffeic acid, ferulic acid and isoferulic acid can be attached to the glucose residue through both glycosidic and ester linkages[1,4]. The number of the sugars generally ranges from 1 to 3 but occasionally 4-sugar residues are also found. According to the number of the sugars linked with hydroxyphenylethyl moieties (Fig. 1), PhGs are categorized into phenylethanoid- monosaccharides, -disaccharides, and -trisaccharides.

The -OH group at the 3- and 4- positions of the aromatic ring can be substituted with 1 or 2 hydroxyl and/or hydroxymethyl substituents[4] that can be modified by other groups, *e.g*., glucose. All the -OH groups in the sugars and the aromatic acids can be linked with other sugar or phenylethyl substitutes or condensed with other groups forming different moieties, such as 1,4-dixoane, seroiridoid, and leading to diverse compounds[4]. Specific chemical structures of PhGs found in plantasiosides, oleoacteosides, and oleoechinaacosides are shown in Fig. 1.

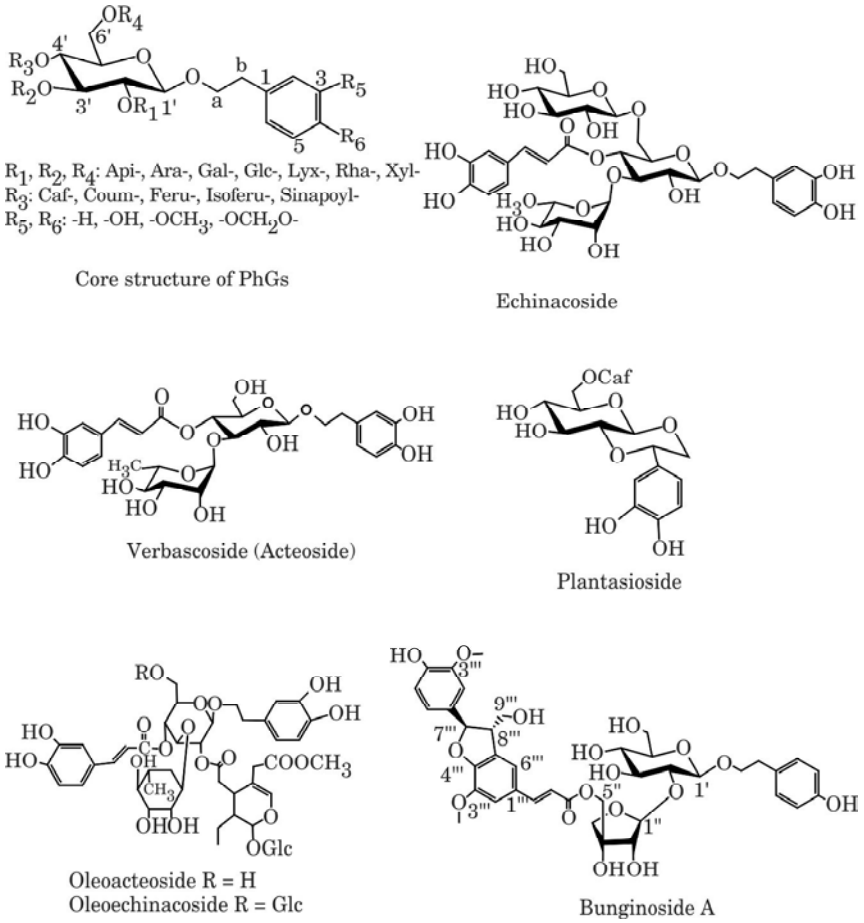

Fig. 1: Chemical structure of PhGs Api-, Apiose; Ara-, Arabinose; Gal-, Galactose; Glc-,Glucose; Lyx-, Lyxose; Rha-, Rhamnose; Xyl- Xylose; Caf-, Caffeoyl; Coum-, Coumaroyl; Feru-, Feruloyl; and Isoferu-, Isoferuloyl.

Physical Property

Most pure PhGs are white, buff or yellow amorphous powders of high polarity and are soluble in polar solvents such as water, methanol, ethanol, and ethyl acetate but insoluble in non-polar organic solvents[27]. All the PhGs show a strong ultraviolet (UV) absorption which makes it easy to monitor these compounds with UV spectrophotometer. The specific UV spectra of each PhGs can also serve as the index to deduce the structure and assign their chemical composition. For example, the UV absorption peaks of acteoside are 232, 246, 289, 332 nm, and that of isoacteoside and echinacoside are 232, 246, 286, 328 nm[28] and 236, 288, 330 nm[29], respectively.

Chemical Properties

As described above, PhGs contain at least one hydoxyphenylethyl moiety and one sugar. Hydroxyl groups in the aromatic acids and sugars enable most of the PhGs to show ferric-reducing antioxidant power (FRAP)[21,30,31]. When incubated with ferric chloride solution (0.1%, w/v) and potassium ferricyanide (1%), or TPTZ (2,3, 5-triphenyl-2H-tetrazolium, chloride), colored $Fe(CN)_2$ or TPTZ-Fe^{2+} complex can be monitored spectrophotometrically at 700 nm or 593 nm respectively[21,30,31]. A FRAP assay is usually applied to determine the antioxidant capability of PhGs.

Isolation, Preparation and Structure Elucidation

Water with certain percentages of polar organic solvents, such as ethanol, methanol, n-hexane, chloroform, ethyl acetate, n-butanol, and petroleum ether, are commonly used to extract PhGs from plant material[19,28,32–36]. In general, dried plant material is extracted several times successively in various polar solvents to obtain crude extract, which is commonly subjected to four chromatographic purification methods, as summarized below.

Isolation and Preparation Techniques

Macroporous resins column chromatography for large-scale preparation of PhGs[37]

Macroporous resin (MR) is a commonly used adsorption matrix. Due to its low cost, high efficiency, easy recycling, and simple scale-up performance, MR has been extensively used for column chromatography. Based on the selective adsorption and filtering effects of MR on different components, natural products can be separated and purified by selecting appropriate adsorption and desorption conditions.

The first step of this separation method is to select the most suitable resin for the target compounds. For separation of the PhGs (mainly contain echinacoside and acteoside) from *Cistanche deserticola*, HPD300 resin is selected for optimal separation efficiency[37]. The separation process including the elution solvent and temperature on columns packed with HPD300 resin is then optimized. Liu *et al*.[37] increased the content of echinacoside and acteoside in the product from 1.8% and 1.4% in the crude extracts to 16.7% and 15.2%, with 80.4% and 90.2% of recovery yields, respectively, demonstrating that MR was efficient for the large-scale production of PhGs from *C. deserticola*. However, the purity of total PhGs was only 76.6%[37] and might be further improved by combining other separation techniques.

High-speed counter-current chromatography (HSCCC)

High-speed counter-current chromatography (HSCCC) is a support-free liquid–liquid partition chromatography technique that is based on the partitioning of compounds between two immiscible liquid phases without a supporting matrix. This eliminates irreversible adsorption on solid supports widely used in the preparative separation of natural products. MR column, multilayer coiled column, or other preparative coils can be applied in combination with HSCCC[28,34,38]. Successful separation by HSCCC largely depends on selecting a suitable two-phase solvent system, usually with partition coefficient values (K) of each target component from 0.5 to 2[34].

By using a HSCCC combined with MR column[38], five PhGs (forsythoside B, verbascoside, alyssonoside, isoverbascoside, and leucosceptoside B) can be isolated and purified from *Lamiophlomis rotata* with the two-phase solvent system composed of ethyl acetate/n-butanol/water (13:3:10, v/v/v) in a one-step separation within 4 h. The recoveries of the five PhGs were 74.5, 76.5, 72.5, 76.4, and 77.0%, respectively.

Applying preparative HSCCC with the fraction enriched through a silica gel column, Han *et al.*[34] isolated and purified four PhGs (purity >95%), acteoside, isoacteoside, syringalide A 3' -α-L-rhamnopyranoside and 2' -acetylacteoside from *Cistanche deserticola* extract in one-step separation with a two-phase solvent system composed of ethyl acetate/n-butanol/ethanol/water (40/6/6/50, v/v/v/v). These studies demonstrate that HSCCC is a very powerful technique for rapid preparative separation and purification of complicated bioactive components from plant materials. It offers excellent sample recovery and yields a sufficiently large amount, high purity compounds which may be supplied as reference substances for chromatography or for bioactivity studies[28,34,38].

Preparative or semi-preparative high-performance liquid chromatography

Preparative or semi-preparative-HPLC is the most widely used technique to simultaneously separate and isolate diverse PhGs constituents from plants[1,10,11,27,32,39–43]. A reversed-phase C18 column with aqueous methanol or aqueous acetonitrile as mobile phase is generally employed and the optimized mobile phase is the most important factor affecting separation efficiency.

In most cases, preparative or semi-preparative-HPLC is used as the final purification step following silica gel chromatography or other separation step. For example, to analyze the metabolites from the stems of cultured *Cistanche deserticola*, crude plant extracts are first subjected to a silica gel CC (200–300 mesh, 1.35 kg) chromatography to obtain 10 fractions.

These 10 fractions were then each subjected to semi-preparative HPLC. Using this approach, 5 new PhGs, and cistanosides J–N, were isolated for further chemical structure identification[41]. In the studies on active compounds from *C. tubulosa*, echinacoside, cistanoside A, cistantubuloside A, acteoside, isoacteoside, 2´-acetylacteoside, and tubuloside A were simultaneously isolated and purified by prep-HPLC, each at over 96.3% purity as determined by HPLC[44]. Some novel PhGs were also obtained using this method, *e.g*. the new PhGs, taraffinisoside A from *Tarphochlamys affinis* (Griff) Bermek[27], isoforsythiaside from *F. suspensa* (Thunb.) Vahl[10], bunginoside A and 3´´,4´´-di-*O*-acetylmartynoside from *Clerodendrum bungei*.[42].

Activity-assay-guided HPLC purification

Activity-assay-guided HPLC purification combines an activity assay with a purification procedure and better affords target compounds having desired bioactivities. A 1,1-diphenyl-2-picryl hydroxyl (DPPH) radical scavenging activity assay[21,35,45], a direct calcineurin assay[46], and an *in vivo* anti-inflammatory activity assay in a mouse model[36] have been used in the isolation of PhGs from plants.

Crude extract is separated into several groups after passing through DIAION HP20 column and silica gel column. Radical scavenging activity is next performed with a rapid thin layer chromatography (TLC) screening method using 0.2% DPPH in methanol. Thirty minutes after spraying the active compounds appear as yellow spots against purple background. The fraction exhibiting bioactivity is then subjected to RP-HPLC for purification. Following this procedure, Abdel-Mageed *et al* obtained 7 active PhGs, including a new one, tocomoloside, 6´-*O*-acetyl verpectoside B. All 7 compounds, acteoside (verbascoside), 6´-*O*-acetylverbascoside, verpectoside B (crassoside), tocomoloside, luteoside A, isoacteoside (isoverbascoside), luteoside B displayed promising antioxidant activity by DPPH assay[45].

Calcineurin is an important regulator of T-cell mediated inflammation. Calcineurin inhibition represents a potential mode of action when investigating the immunomodulatory activity of CPG-containing plants. Direct calcineurin assay-guided fractionation of *Teucrium chamaedrys* and *Nepeta cataria* led to the isolation of the CPGs, teucrioside, verbascoside and lamiuside A (teupolioside). These three compounds inhibit calcineurin both in the presence and absence of calmodulin, suggesting a direct interaction with calcineurin[46].

The *in vivo* anti-inflammatory activity of the extracts has been assessed using a carrageenan-induced hind paw edema model in mice. Through this bioassay-guided fractionation and isolation procedures, ajugol, aucubin, lasianthoside I, catalpol, ilwensisaponin A and C and verbascoside were

isolated from *Verbascum mucronatum* Lam. Verbascoside was found to possess significant wound healing activity as well as antinociceptive and antiinflammatory properties[36].

Profiling and Structure Elucidation

HPLC and ultra-high-performance liquid chromatography (UPLC) are the most direct and rapid ways to identify and quantitatively analyze known compounds using authentic standard compounds. The overall analytical procedure is fast, accurate and suitable for the quantitative analysis of a large number of samples. For example, 5 PhGs, acteoside, ligupurpuroside A, isoacteoside, lipedoside A-I, and ligupurpuroside B were simultaneously determined in small-leaved *Kudingcha* by UPLC using a photo diode array (PDA) detector[47]. Unfortunately, in many cases, not all the standard compounds are available making identification of unknowns quite difficult based solely on retention time and PDA detection.

Electrospray ionization-mass spectrometry (ESI-MS)

ESI coupling with tandem mass spectrometry (ESI-MS/MS) has been a powerful tool to characterize unknown constituents in plants. The molecular mass of a series of molecular ions or fragments produced by ESI can be detected and the fragmentation pathway can be proposed according to the characteristics of these fragments, from which a putative compound structure can be deduced. ESI-MS/MS is a sensitive technique and only requires a small amount of sample and the compounds do not need to be purified, making ESI-MS widely used in profiling natural products. HPLC and UHPLC are usually coupled with ESI-MS to further enhance this method of identification of natural products.

HPLC-ESI-MS/LC-ESI-MS

HPLC-ESI quadrupole time-of-flight tandem mass (ESI-QTOF MS/MS), was employed on Radix *Scrophulariae* to identify eleven PhGs constituents based on their retention times, accurate mass of their molecular-ion [M-H] and productions of the MS/MS spectra. Eight compounds were identified from their QTOF MS/MS data, which afforded sufficient structural information for their full structural elucidation. A total of 36 compounds were identified or tentatively characterized, demonstrating HPLC-ESI-QTOF-MS/MS as an important technique for the rapid and sensitive structural elucidation of complex constituents of natural products, such as those obtained from Radix *Scrophulariae*[48]. In another report, 63 phytochemicals including nine unusual PhGs were tentatively characterized from *Globularia alypum* L. leaves by LC-ESI-QTOF-MS[49].

UHPLC/ESI–QTOF–MS/MS

On the basis of parention scanning for m/z 161 (Fig. 2), the characteristic production for PhGs, Qi *et al*. established a target profiling analysis approach using UPLC/ESI–QTOF–MS/MS[50]. This method was successfully employed to discriminate the chemical composition of PhGs between *Plantaginis herb*a and *Plantaginis semen*. In total, 34 PhGs were characterized and identified from their retention times, MS, and tandem quadrupole MS/MS data.

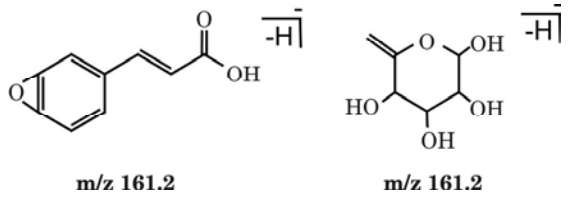

Fig. 2: Characteristic product ion for PhGs

Based on the UPLC/ESI–QTOF–MS/MS method, the important structural information on the types of aglycone and saccharide sequences present can also be obtained. Combined with the HPLC retention behavior, proposed fragmentation pathways based on literature data provide for the tentative identification of 13 PhGs in a crude extract of *C. deserticola* using high-resolution (HR) MS and MS/MS spectra. This success suggests that UPLC/ESI–QTOF–MS/MS is a rapid and accurate method to profile and identify PhGs in the crude extracts[51].

Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) spectroscopy can facilitate the elucidation of the structure of almost any unknown natural product. Increasing sophistication in NMR spectroscopy has led to the extensive use of this technique for the structure elucidation of PhGs, particularly ^{13}C and two-dimensional NMR spectroscopy[1]. The identification of a new PhG, bunginoside A (Fig. 1), from *Clerodendrum bungei* Steud. represents an example of how to use this technique, combined with HR-ESI-MS and NMR can be used to elucidate PhG structure[42]. The molecular formula was assigned as $C_{39}H_{46}O_{17}$ by HR-ESI-MS at *m/z* 785.2696 ([M-H]$^-$, calcd. for 785.2657), and confirmed by analysis of the ^{13}C NMR spectroscopic data. The ^1H and ^{13}C NMR spectroscopic data exhibited signals arising from: three benzene rings [an AB coupling system at δ_H 7.04 (2H, d, J = 8.5 Hz), and δ_H 6.66 (2H, d, J = 8.5 Hz); an ABX coupling system at δ_H 6.97 (1H, d, J =1.6 Hz), δ_H 6.85 (1H, dd, J = 8.2, 1.6 Hz), and δ_H 6.80 (1H, d, J = 8.2 Hz); an AX coupling system at δ_H 7.17 (1H, brs), and δ_H 7.11 (1H, brs)]; one trans-C=C bond [δ_H 7.69 (1H, d, J = 15.9 Hz), and δ_H 6.43 (1H, d, J = 15.9Hz)]; one

β-glucopyranosyl moiety [anomeric proton at δ_H 4.35 (1H,d, J = 7.8 Hz), and carbon resonances at δ_C 103.1 (C-1′), 77.9 (C-2′), 79.0 (C-3′), 71.8 (C-4′), 77.9 (C-5′), and 62.7 (C-6′)]; one β-apiofuranosyl moiety [anomeric proton at δ_H 5.46 (1H, brs), and carbon signals at δ_C 110.0 (C-1′′), 78.4 (C-2′′), 79.2 (C-3′′), 75.5 (C-4′′), heteronuclear multiple bond correlation spectroscopy (HMBC) and 68.5 (C-5′′)], assigned by heteronuclear single quantum correlation spectroscopy (HSQC), and ^{1}H–^{1}H correlation spectroscopy (COSY) spectra, respectively. Acidic hydrolysis of this compound afforded D-glucose and D-apiose as component sugars. The above spectroscopic characteristics resembled those of glypentoside C from reference. However, distinct from glypentoside C, HMBC correlations were observed between: H-1′ (δ_H 4.35) and C-8 (δ_C 71.8); H-7 (δ_H 2.81) and C-1 (δ_C 130.5), C-2 (δ_C 131.0), and C-8 (δ_C 71.8); an δ_H-2′ (δ_H 3.42) and C-1′′ (δ_C 110.0). These indicated that the aglycone of the compound was tyrosol, but not methoxyquinol, and it was thus a tyrosol apiosyl-(1→2)-glucopyranoside. As for glypentoside C, a dihydrobenzo[b]furan neolignan moiety, could also be established by ^{1}H–^{1}H COSY correlations from H-7′′′′ (δ_H 5.60) through H-8′′′′ ((δ_H 3.56) to H-9′′′′ ((δ_H 3.86), in combination with HMBC correlations between H-7′′′′ (δ_H 5.60) and C-4′′′′ (δ_C 152.1), C-1′′′′ (δ_C 134.1), C-2′′′′ (δ_C 110.6), and C-6′′′′ (δ_C 119.9); between H-8′′′′ (δ_H 3.56) and C-4′′′′ (δ_C 152.1), and C-5′′′′ (δ_C 131.0), and between H-7′′′′ (δ_H 7.69) and C-2′′′′ (δ_C 113.7), C-6′′′′ (δ_C 119.2) and C-9′′′′ (δ_C 169.0). The relative configuration of C-7′′′′ and C-8′′′′ in the dihydrobenzo[b]furan neolignan moiety was determined to be in the *trans* form by NOESY interactions between H-7′′′′ (δ_H 5.60) and H-9′′′′ (δ_H 3.86), whereas the absolute configuration of the dihydrofuran ring was determined by circular distoism (CD) spectroscopy. Its CD spectrum showed a positive Cotton effect at 287 nm, indicating that the configuration of bunginoside A must be 7′′′′ R and 8′′′′ S according to literature (Fig. 1). The NMR signals of H-5′′′′ and C-5′′′′ were shifted upfield to δ_H 4.33 and δ_C 68.5, showing C-5′′′′ was esterified with the dihydrobenzo[b]furan neolignan moiety. Additionally, NOESY interactions between 34 -OMe (δ_H 3.92) and H-24 (δ_H 7.11); and between 3′′′′ -OMe (δ_H 3.85) and H-2′′′′ (δ_H 6.97) could also be observed, indicating the methoxy groups were located at C-3′′′′ and C-3′′′′, respectively. Based on the above, structure of the compound was elucidated as (4-hydroxy-3-methoxyphenyl)-ethxoyl 2-*O*-[5-*O*-[(2*E*)-3-[(2*S*,3*R*)-2,3-dihydro-2- (4-hydroxy-3-methoxy-phenyl)-3-(hydroxymethyl)-7-methoxy-5-benzofuranyl]-1-oxo-2-propenyl]-β-D-apiofuranosyl]- β-D-glucopyranoside, with a common name bunginoside A (Fig. 1).

Capillary Electrophoresis

Capillary electrophoresis (CE) is an electrokinetic separation method performed in submillimetre capillaries and in microfluidic and nanofluidic channels. Due to the high separation efficiency, CE became a powerful tool

to analyze multiplex ingredients in plants and was also employed to display the profiling of PhGs.

The separation efficiency of CE largely depends on separation conditions. Usually, the working potential, pH value, ionic strength of running buffer, separation voltage, and sample injection time would all affect the separation efficiency and should be optimized before running samples. Dong *et al.*[52] developed an efficient optimization method by adding 5.0 mmol L^{-1} of β-cyclodextrin (β-CD) modifier into the running buffer to improve the analytical resolution and sensitivity for natural products. The β-CD acts as chelating agent, forming complexes with analytes, decreasing their net negative charge and altering their electrophoretic mobilities leading to improved separation in CE. Applying the established capillary zone electrophoresis (CZE) method with ultraviolet (UV) detection, Dong *et al.* simultaneously separated four PhGs and aglycones (homovanillyl alcohol, hydroxytyrosol, 3,4-dimethoxycinnamic acid, and caffeic acid) in *Lamiophlomis rotata* and *Cistanche*.

CE-microemulsion electrokinetic chromatography (MEKC) is another technique of CE. With the optimized surfactant concentration, background electrolyte, pH, electrolyte concentration, and buffering capacity, the iridoid glycosides aucubin and catalpol, and the PhGs acteoside and plantamajoside from water extracts of *Plantago lanceolata*, *Plantago major*, and *Plantago asiatica* were simultaneously determined and quantified within 20 min using CE-MEKC[53].

BIOACTIVITY AND STRUCTURE-ACTIVITY RELATIONSHIP

Most PhGs are derived from medicinal herbs traditionally used to treat liver disorders, fever, asthma, jaundice, and inflammatory- immune-, neuro-related diseases, which have attracted chemists to mine those compounds and discover their potential clinical applications. The reported biological activities of PhGs include antioxidant[30], antibacterial[10], immuno-modulatory[46], antiinflammatory[54], anti-HBV[27], antiproliferation[14], antitumor[4], neuroprotective[21], and hepatoprotective[55]. Such activities make these compounds valuable and promising drug or drug-precursor candidates. This section focuses on the potential clinical use activities of PhGs, as well as the chemical structure-activity relationship or pharmaceutical mechanism of those compounds.

Antioxidant and Free Radical Scavenging Capability

Oxidation produces free radicals, which start chain reactions in cells. If free radicals are overproduced, these chain reactions can cause damage or death to the cells leading to physiological disorders. Antioxidants terminate these

chain reactions by removing free radical intermediates and inhibit other oxidation reactions by they themselves being oxidized, thus benefiting human health. Most of the PhGs such as acteoside (verbascoside), isoverbascoside, forsythoside B, leucosceptoside B, poliumoside B, plantamajoside, desrhamnosyl, calceorioside B, lianqiaoxinoside B, nuomioside A, and isonuomioside A, are antioxidant and can scavenge free radicals,[10,15,21,27,31,33,56–60].

Seven PhGs, acteoside (verbascoside), 6´-*O*-acetylverbascoside, verpectoside B (Crassoside), 6´-*O*-acetyl-verpectoside B (tocomoloside), luteoside A, isoacteoside (isoverbascoside), luteoside B and one iridoid, ixoside were isolated from *Tecoma mollis*. All PhGs, with the exception of ixoside, showed promising antioxidative activity based on DPPH free radical scavenging assay[45]. The antioxidative effects of these compounds were correlated to the number of free phenolic hydroxyl groups in the form of 3,4-dihydroxy (catechol) moiety in their structures, which explains the similarity of their antioxidant activities. The blocking of the hydroxyl groups by a methyl group, as a methyl ether, leads to a dramatic decrease of the antioxidant activity[61].

Poliumoside B and poliumoside are PhGs with an (E)-caffeoyl and 3,4-dihydroxyphenylethyl moieties linked at C-4´ and C-1´ of the glucosyl unit. The most significant difference between them is that poliumoside B is a phenylethanoid tetrasaccharide with an additional arabinosyl unit. Both poliumoside B and poliumoside showed higher DPPH radical-scavenging activities (EC_{50} of 4.8 and 7.0 μM, respectively) than all the other flavonoid glycosides. The mechanism of the radical-scavenging activity of flavonoids is thought to be through hydrogen atom donation and these two poliumosides have several free hydroxyl groups that can act as hydrogen donors. Additionally, these two PhGs possess the (*E*)-caffeoyl residue, the C7–C8 unsaturation and the C9-keto group responsible for the co-planarity and for the electronic delocalization throughout the molecule, leading to the stabilization of the aryloxy radical. The greater antioxidant activity than flavonoid glycosides could be attributed to the presence of a second phenolic ring with an ortho-dihydroxy group[56].

Other effects or pharmaceutical activities of PhGs are due to their significant antioxidant activity, *e.g.*, prevention effects of myocardial ischemia injury on coronary artery disease[63].

Antioxidant, Anti-inflammatory and Neuroprotective Properties

Oxidative stress has been considered as a major cause of cell damage in neurodegenerative disorders. *In vitro* experiments revealed that isocampneoside II (ICD) from hardwood genus *Paulownia*, eliminated approximately 81% superoxide radical at the concentration of 0.1 mg/ml,

showed strong reducing power, and provided protection against oxidative protein damage induced by hydroxyl radicals. Pretreatment of PC12 cells with ICD prior to hydrogen peroxide exposure elevated cell viability, enhanced activity of superoxide dismutase and catalase, and decreased levels of malondialdehyde and intracellular reactive oxygen species (ROS). This ICD pretreatment also inhibits cell apoptosis and Bax/Bcl-2 ratio induced by hydrogen peroxide. Structures with free hydroxyl groups, especially the *E*-caffeoyl and the 3,4-*O*-dihydroxyl catechol in phenylethyl units in ICD act as efficient free radical scavengers, thus ICD readily reacts with free radicals to stabilize and terminate the radical chain reactions. This finding suggests that ICD might be considered as a potential antioxidant agent for neurodegenerative diseases[21].

Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by deposits of amyloid fibrils, mainly consisting of 40-mer and 42-mer amyloid β-proteins (Aβ40 and Aβ42). Acteoside isolated from *Orobanche minor* strongly inhibited the aggregation of Aβ42 in a dose-dependent manner. The structure-activity relationship for acteoside and its derivatives indicated that two catechol moieties present at a position a little distant through a glucose moiety prove to be indispensable for inhibition of Aβ42 aggregation. The catechol moiety in the PhGs is essential for the inhibition. The auto-oxidation of the catechol moiety in the caffeoyl group might be related to the mechanism for the inhibition. Such covalent modification may destabilize the β-sheet structure in amyloidogenic polypeptides[64].

Parkinson's disease (PD) is another degenerative disorder of the central nervous system. Inflammatory of neuro cells may cause PD. The antiinflammatory activity evaluation, of 5 new PhGs and 15 known ones isolated from *Cistanche deserticola,* revealed that cistanosides K and tubuloside B show potent inhibitory effect on the lipopolysaccharide (LPS)-induced nitric oxide production in mouse microglial cells (BV-2 cells), which indicates that PhGs might exert potential inhibitory effects on microglia-involved neuroinflammation, resulting in neuroprotection in inflammation related neuronal degenerative diseases including AD and PD[41].

Based on the primary study, the structure-activity relationship of these PhGs indicated that: (1) the 8-*O*-β-D-glucopyranosyl part having the 6´-*O*-caffeoyl or feruloyl group showed stronger activity than that having the 4´-*O*-caffeoryl or feruloyl group; (2) the aglycone having the 4-hydroxy-3-methoxyphenyl group showed stronger activity than that having the 3,4-dihydroxy group; (3) the 6´-*O*-caffeoyl group showed stronger activity than that having the feruloyl group; (4) introduction of a glucose moiety at 3´´´ led to the decrease of activity, while the 6´-*O*-caffeoyl group having the 3´´´ -*O*-β-D-glucopyranosyl showed stronger activity than that having the 3-methoxyl group; (5) the 6´-*O*-β-D-glucopyranosyl moiety reduced the activity

(13 > 17); and (6) introduction of the 2´-*O*-acetyl moiety increased the activity. Understanding the effect of these moieties on the activity may help in the development of new therapeutic drugs.

Other PhGs, such as echinacoside isolated from *Cistanche salsa,* are probably novel, orally active, non-peptide inducers of neurotrophic factors (NTFs) and also has the potential to prevent or halt the progress of neurodegeneration in PD[62].

Inhibition of Aldose Reductase (AR) and α-Glucosidase

Aldose reductase (AR) inhibitors can inhibit abnormally elevated levels of sorbitol in many organs of patients with diabetes. AR inhibitors have considerable therapeutic potential against diabetic complications and do not increase the risk of hypoglycemia. In the evaluation of the inhibitory activities PhGs and phenolic compounds from *Paulownia coreana* against recombinant human aldose reductase (rhAR) and sorbitol formation in human erythrocytes, Kim *et al*. found that PhGs were more effective than the phenolic compounds and ICD significantly inhibited rhAR with an IC_{50} value of 9.72 mM and inhibited sorbitol formation in a rat lens incubated with a high concentration of glucose. The inhibition of rhAR was uncompetitive. This result indicates that ICD is effective in either preventing or retarding sugar cataract formation associated with diabetes may have a potential therapeutics against diabetic complications[65]. Echinacoside and acteoside also demonstrate potent rat lens AR inhibitory activity[66]. The inhibitory activity of PhGs against rhAR appears to be related to the presence of the dihydroxyphenylethyl moiety, steric hindrance, and the location of the hydroxyl group[65].

The α-glucosidase inhibitor was used to treat diabetes by inhibiting the α-glucosidase from breaking down the polysaccharide or disaccharide chain, thereby decreasing the level of glucose in blood. Three principal PhGs, verbascoside, leucosceptoside A and isoacteoside, isolated from *Clerodendrum bungei* exhibited stronger inhibitory effects against α-glucosidase than the positive control acarbose, indicating that those PhGs may have potential therapeutic use for the treatment of diabetes[42].

Antiinflammatory and Hepatoprotective Property

Since liver damage usually occurs with cell or tissue inflammation, antiinflammatory agents have been used as hepatoprotective drugs. 3-(4,5-Dimethylimzol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay of PhGs constituents on D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes revealed that echinacoside (IC_{50} = 10.2 µM), acteoside (IC_{50} = 4.6 µM), isoaceteoside (IC_{50} = 5.3 µM), 2´-acetylacteoside (IC_{50} = 4.8 µM), tubulosides A (IC_{50} = 8.6 µM) and B11 (IC_{50} = 14.6 µM), and kankanoside

G11 (IC_{50} = 14.8 μM) showed strong inhibitory effect. By analyzing their chemical structures and activities, the role of moieties in PhGs contributing to their inhibitory activity were deduced. The aglycone portion was essential for the activity; the aglycone having the 3,4-dihydroxy group showed stronger activity than that having the 4-hydroxy group; the 8-*O*-β-D-glucopyranosyl part having the 4´-*O*-caf-feoyl group showed stronger activity than that having the 6´-*O*-caf-feoryl group; while the 6´-*O*-β-D-glucopyranosyl moiety and introduction of the 2´-*O*-acetyl moiety reduced the activity. These 5 PhGs also reduced tumor necrosis factor (TNF)-α-induced cytotoxicity in L929 cells and exhibited hepatoprotective effects *in vivo*[55], indicating they had hepatoprotective activity.

The extracts, preparations, and pure verbascoside from *Harpagophytum procumbens* also exhibited strong antiinflammatory properties related to nitric oxide and cytokine production (TNF-α and IL-6), and COX-1 and COX-2 expression by macrophages, which suggested that the potential as antiinflammatory agents may be due to their strong anticomplement activity[67]. Verbascoside was also found to possess significant wound healing activity as well as antinociceptive potentials without inducing any apparent acute toxicity or gastric damage[36]. Some newly found PhGs, leunoside E, leunoside F[68], and taraffinisoside A[27] had hepatoprotective and anti-HBV activities as reported.

Antiinflammatory and Immunomodulating Activity

Calcineurin is an important regulator of T-cell mediated inflammation. Calcineurin inhibition should be considered as a potential mode for investigating the immunomodulatory activity of CPG. Activity-guided fractionation of *Teucrium chamaedrys* and *Nepeta cataria* led to the isolation of the CPGs teucrioside, verbascoside and lamiuside A (teupolioside). The three compounds inhibited calcineurin both in the presence and absence of calmodulin, suggesting a direct interaction with calcineurin[46].

Chemokines and cytokines are mediators crucially implicated in the recruitment and local activation of immune cell populations during inflammatory events in the skin. The antiinflammatory effects of *Verbascum xanthophoeniceum* crude extract, its fractions, iridoid glycosides, and PhGs in primary cultures of normal human keratinocytes (NHK) were investigated, which revealed that PhGs, verbascoside and forsythoside B effectively inhibited the expression of spontaneous and interferon (IFN)-γ-triggered pro-inflammatory chemokines at transcriptional and translational levels at a dose-dependent pattern. The glycosidic moiety appears to be crucial for IL-8 but not for MCP-1 or IP-10 inhibition. These substances could be considered as potential active components for topical compositions aimed at the regulation of chronic inflammatory skin disorders such as psoriasis and atopic dermatitis[69].

The antiinflammatory activity of PhGs was also used to treat other diseases. Phenylethanoid acteoside ameliorated intestinal inflammation in dextran sulfate sodium-induced colitis[70] and provided significant gastric protection in an acute ulcer induction model and topical antiinflammatory activity in a mouse ear edema model[71].

Antiproliferation and Antitumor Properties

Acteoside has significant effect on inhibition the proliferation of prostate cancer PC-3 cells[14]. Acteoside also exhibits inhibition on the proliferation of HL-60 leukemia cells[72] and inhibits phorbol myristate acetate (PMA)-induced invasion and migration of human fibrosarcoma cells through Ca^{2+}-dependent CaMK/ERK and JNK/NF-κB-signaling pathways[73]. These results demonstrate that acteoside has general inhibition capacity on different cancer cell lines and might be a potent anticancer agent in therapeutic strategies.

The cinnamic acid moiety of some plant metabolites, such as curcumin and caffeic acid phenethyl ester, are believed to play important role in the antiproliferation activities on cancer cell lines[74]. The antiproliferation effect of acteoside shows nearly double the potency of echinacoside and calceolarioside. The α-Rha-(1→3)-Glc disaccharide unit and the 4-caffeoyl function in acteoside were proposed to contribute to the higher antiproliferation activity[14].

In vitro cytotoxic potential against mouse skin melanoma cancer cell line KML show that echinacoside (75%), cistanoside A (33%), cistantubuloside A (83%), acteoside (81%), and 2´-acetylacteoside (93%) all inhibited the tumor growth at different degree ranging from 33% to 93%[44]. All these findings indicate that PhGs are potentially valuable compounds as anticancer candidate drugs.

Antiviral, Antibacterial and Antiprotozoal Properties

A number of PhGs have been reported to have antiviral activities. Acteoside and acteoside isomer showed potent inhibitory activities against HIV-1 integrase with IC_{50} values of 7.8 ± 3.6 and 13.7 ± 6.0 μM, respectively[75]. In an HIV gp41 binding affinity assay, calceolarioside B and esculetin showed moderate binding affinities with HIV gp41 giving IC_{50} values of 0.1 mg/ml and 0.5 mg/ml, respectively[76].

Total PhGs from *Monochasma savatieri* Franch. ex Maxim (TPG) show significant bactericidal activity against *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* or *Escherichia coli*, prolonged survival rates of mice with *Pseudomonas aeruginosa* or *Staphylococcus aureus* infection-induced sepsis, and reduce the bacterial colony-forming units in lung tissue.

TPG also significantly reduce xylene-induced ear edema and granuloma formation, induced by cotton pellet, in a dose-dependent manner. In addition, administration of TPG (1.5 g/kg) for 15 days did not result in liver, kidney, spleen and thymus toxicity in rats. These results indicate that TPG might be useful for the development of a novel treatment in respiratory infections or pneumonia caused by *P. aeruginosa* or *S. aureus*[77].

Pure PhGs compounds also exhibit antibacterial activity. Among the glycosides tested, forsythoside B and verbascoside (acteoside) show considerable antibacterial activities against all strains of *S. aureus* with the minimum inhibitory concentration (MIC) values ranging from 64 µg/l to 256 µg/l, comparable to a positive control, suggesting that forsythoside B and verbascoside as promising new antimicrobial drugs[40]. Analysis of the structure-activity relationship indicates that despite being structurally closely related, phlinoside C did not show any activity against any of the strains at the test concentrations. The introduction of the third sugar, rhamnose, in forsythoside B might have contributed to its inactivity against any of the test strains[40].

Another exploited property of acteoside is as a potent synergistic compound in combination with amphotericin B (AmB) against selected pathogenic species[78]. Acteoside alone exhibited no intrinsic antifungal activity but the combination of acteoside at 3.12 and 12.5 mg ml^{-1}, with sub-inhibitory concentrations of AmB, it results in potent fungicidal effect and also exhibits significantly extended post-antifungal effects, demonstrating that acteoside significantly reduces the inhibitory concentration of AmB against the clinically important fungal pathogens, *Cryptococcus neoformans*, *Candida species* and *Aspergillus* species[78]. The antioxidant activity of acteoside may contribute to this synergistic action of AmB. The sub inhibitory concentration of AmB probably enhances the cellular uptake of acteoside and, once inside the cell, this compound may inhibit one or more physiological or biochemical process. Another possibility is that antioxidants protect AmB from auto-oxidation, thereby decreasing the rate of its spontaneous inactivation and prolonging its biological activity[78].

Antiprotozoal activity is another property of PhGs. Among all the isolated compounds including one iridoid and seven PhGs from *Tecoma mollis*, luteoside B and luteoside A shows the highest antileishmanial activity with IC_{50} values of 6.7 and 15.1 µg/ml, respectively. Luteoside A also exhibited moderate antimalarial activity (45% inhibition) against chloroquine sensitive (D6) clones of *Plasmodium falciparum*[45], suggesting that PhGs might also be explored as antimicrobial drugs.

Antinutritional Properties

The finding of antinutritional properties of PhGs resulted from the investigation of the function of Kudingcha (bitter tea), which is a well known

Chinese traditional drinking tea and has long been used in folk medicine as a diuretic and slimming agent, and for the treatment of hypertension, sore throat as well as inflammation[79]. The PPGs, including acteoside, osmanthuside, ligupurpuroside A, ligupurpuroside B, and ligupurpuroside C are important ingredients in this plant.

The antinutritional properties of PPGs have been explored by investigating the *in vitro* inhibitory effects on typical digestive enzymes pepsin, trypsin and α-chymotrypsin, using multi-spectroscopic method and docking studies. The study showed that the IC_{50} values for PPGs inhibiting pepsin, trypsin and α-chymotrypsin activity were 0.68, 0.38 and 0.42 mg/mL, respectively, which indicate that PPGs inhibit the activity digestive enzymes. This inhibition may be due to the interaction between the compounds and the enzymes. Reaction kinetics suggests that the inhibition is non-competitive. The positive ΔH and ΔS values in this interaction suggest that the hydrophobic interactions may play a major role in the binding of PPGs to digestive enzymes. The negative ΔG values suggest that the binding of PPGs with the digestive enzymes is spontaneous.

The pepsin–PPG, trypsin–PPG and α-chymotrypsin–PPG complexes have also been characterized by CD. With PPG treatment, changes in spectra were observed. After PPG binding, the secondary structures of the complexes, α-helical and β-sheet contents decreased and the unordered structure content increased, suggesting that the binding effect of PPGs on these digestive enzymes causes the protein to unfold. Docking studies were used to better understand the acteoside-enzyme complexes. The results revealed that acteoside might bind Asp32 and Asp215 of pepsin and Ser195 and His57 of trypsin and α-chymotrypsin. These interactions might impact the optimal combination of these enzymes and their substrates. PPGs may have complexing abilities with digestive enzymes, which lead to reduced digestive enzyme activity in the body[79].

BIOSYNTHESIS AND CHEMICAL SYNTHESIS OF PHGS

Biosynthesis of PhGs

Early studies on acteoside biosynthesis using isotope labeled precursor feeding in *Syringa vulgaris* cell suspension cultures indicate that the caffeoyl group of acteoside is derived from L-phenylalanine, while the 3, 4-dihydroxy-phenylethanol unit was derived from L-tyrosine[80] (Fig. 3). A second isotope labeled precursor-feeding study using *Olea europaea* cell confirmed that the hydroxytyrosol moiety of acteoside is biosynthesized from tyrosine through dopamine, whereas the caffeoyl moiety of acteoside is biosynthesized from phenylalanine through the cinnamate pathway. Dopamine is incorporated into acteoside through oxidation to the corresponding aldehyde, reduction to the alcohol, and then beta-

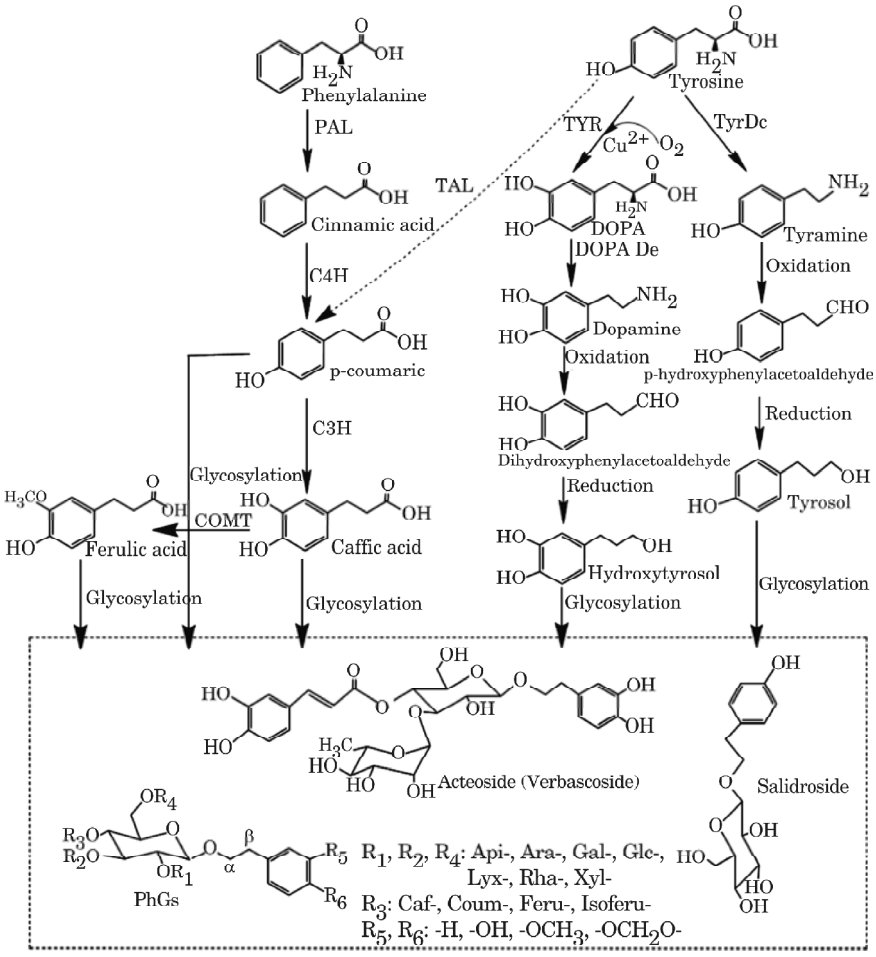

Fig. 3: Putative biosynthesis pathway of acteoside, salidroside and other PhGs C_3H, Cinnamate-3-hydroxylase; C_4H, Cinnamate-3-hydroxylase; COMT, Caffeic acid *O*-methyltransferase; DOPA, L-3,4-Dihydroxyphenylalanine (L-3-Hydroxytyrosine); DOPA Dc, DOPA decarboxylase; PAL, Phenylalanin ammonialyase; TAL, Tyrosin ammonialyase; TYP, Tyosinase; and TypDc, tyrosine decarboxylase.

glycosylation[81]. Precursor feeding with L-phenylalanine, L-tyrosine, sodium acetate and phenylacetic acid at appropriate concentrations enhanced the production of PhGs in the cell cultures of *Cistanche deserticola*. The feeding precursor with L-phenylalanine gave the highest production improvement[82], which further demonstrated the importance of L-phenylalanine and PAL enzyme for the biogenesis of PhGs.

Recently, the biogenetic pathway of forsythoside B and acteoside of *Callicarpa peii* was proposed by H.T. Chang on the basis of the isolated

structure-related PhGs metabolites[83]. Two new trisaccharide intermediates of PhGs, peiioside A1/A2 and peiioside B, structural elucidated as *O*-α-L-rhamnopyranosyl- (1´´→3´)-*O*-[β-D-apiofuranosyl-(1´´´ →6´)] -4´-*O*-[(*E*)-caffeoyl]-D-glucopyranoside] (1a/1b), 3,4-dihydroxy-β-phenylethoxy-*O*-[β-D-apiofuranosyl-(1´´´→6´)-α-L-rhamnopyranosyl-(1´´→3´)-*O*-β-D-glucopyranoside], were isolated from the plant, together with 5 biogenetic relevant known compounds forsythoside B, acteoside, cistanoside F, decaffeoylacteoside, 3,4-dihydroxyphenylethyl-8-*O*-β-D-glucoside. Five related intermediates provided further support for the biogenetic pathway[83].

In another precursor feeding study on the accumulation of PhGs to *C. deserticola* cell suspension culture, the production of salidroside increased with the increasing concentration of tyrosine and the increase was more significantly by feeding with tyrosine than with phenylalanine, suggested that tyrosine is a better precursor for PhGs accumulation compared to phenylalanine and that salidroside, is derived from tyrosine rather than phenylalanine[84]. Tyrosine decarboxylase (TyrDc) was the rate limiting enzymes in the biosynthetic pathway from tyrosine to salidroside. Acteoside in an *O. europaea* cell line is biosynthesized through dopamine from tyrosine[81], which provides evidence that acteoside is not further modified from salidroside. Dopamine is derived from tyrosine and catalyzed by tyrosinase[84]. It can be concluded that the tyrosine branch is also important since the core structure of PhGs maybe derived from tyrosine, but not phenylalanine[84].

Taken together with this literature and the general way of phenylpropanoid metabolism[85], a putative biosynthesis pathway of acteoside, salidroside and other PhGs was proposed (Fig. 3). As example of the biosynthesis of acteoside, the caffeoyl moiety comes from phenylalanine and the hydroxytyrosol moiety is from tyrosine.

Producing PhGs with Biotechnology

Commercially used PhGs are mainly extracted from the wild or from cultivated plants. Plant cell culture or tissue cultures of some plant species have been established to produce high-value compounds.

Devil's claw (*Harpagophytum procumbens*) suspension cell cultures produces the PhGs verbascoside, leucosceptoside A, β-OH-verbascoside and martynoside[67]. Applying a 3-L stirred tank reactor and a 1-L glass-column bioreactor (operated with pulsed aeration), the cell cultures of Devil's claw cell suspension yielded 56 mg of verbascoside/L/day with the stirred tank reactor, similar productivities to those of shake-flask cultures (55 mg verbascoside/L/day) and 165 mg verbascoside/L/day in the pulse-aerated column reactor[86]. Stancheva *et al.*[87] also found that *H. procumbens* cells

could accumulate verbascoside (517 mg l^{-1}), leucosceptoside A (107 mg l^{-1}) and β-OH-verbascoside (80 mg l^{-1}), indicating that cell suspensions cultures are more promising as potential commercial sources of metabolites such as PhGs. Cells from other species as *Cistanche deserticola*[82,84,88–91], *Echinacea angustifolia*[92], *Olea europaea*[81] were also used to produce those compounds.

Adventitious roots and transformed hairy roots have also been used to produce PhGs. Adventitious root cultures of *Castilleja tenuiflora* Benth provide an alternative source of material for production of PhGs. When cultured in B5 medium containing either 10 μM indole 3-acetic acid or 10 μM α-naphthaleneacetic acid. The greatest dry biomass yield (30 gL^{-1}) was achieved at 30 days after transfer of roots into indole 3-acetic acid-containing medium. The highest specific yields of PhGs, the maximum level of verbascoside was 14 mg g^{-1} dry root biomass (438 mg L^{-1}) at 30 days after root transfer, and the maximum yield of isoverbascoside was 37 mg g^{-1} dry root biomass (522 mg L^{-1}) at 23 days after root transfer. This result indicates that adventitious root cultures of *C. tenuiflora* may be a promising system for the scaled-up culturing and production of PhGs[2].

Transformed hairy roots differ from adventitious roots, as transformed hairy roots are generated after *Agrobacterium rhizogenes* transformed and integrated its T-DNA into the plant genome. Transformed hairy root cultures of *H. procumbens* can yield verbascoside, leucosceptoside A, β-OH-verbascoside, and martynoside. The production of the former three PhGs in transformed root cultures is lower than that in suspension cell cultures, yet martynoside is not present in cell cultures[67].

A number of other hairy roots also yield considerable PhGs. For instance, *H. procumbens* Hp-3 root clone produce 8 mg g^{-1} dry wt. of verbascoside and mg g^{-1} dry wt. of isoverbascoside[93]. *Rehmannia glutinosa* RS-2 root line accumulated mg g^{-1} dry wt. of verbascoside and 3.46 mg g^{-1} dry wt. of isoverbascoside[3], *Verbascum xanthophoeniceum* VX1 and VX6 hairy roots accumulated 23.3±0.4 mg g^{-1} and 22.0±0.7 mg g^{-1} of verbascoside, respectively. These root strains also yield three other PhGs, forsythoside B, leucosceptoside B and martynoside[94].

Biotic and abiotic factors, such as chitosan elicitor[89], 2-aminoindan-2-phosphonic acid[90], rare earth elements[91], tyrosine, phenylalanine, caffeic acid, and cucumber juice at proper concentrations[95] can increase the total accumulation of PhGs in these cultures.

Producing PhGs with Chemical Synthesize Method

The significant pharmaceutical activities of PhGs have attracted chemists to synthesize these high-value compounds. The chemical synthesis of two

trisaccharides related to leonoside E and F have been reported[96]. In their synthesis, target oligosaccharides were prepared in the form of their *p*-methoxyphenyl glycosides using a common disaccharide acceptor. The synthesis contains 4 schemes. Scheme 1 was to synthesize the monosaccharide acceptor and donor, while Scheme 2 was used to synthesize a trisaccharide, p-methoxyphenyl α -L-arabinopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside, and scheme 3 was used to synthesize trisaccharide, p-methoxyphenyl- α -L-rhamnopyranosyl-(1→3)-6-*O*- (β-D-glucopyranosyl)-β-D-glucopyranoside, having a different configuration from the trisaccharide synthesized in scheme 1. All reaction steps were high yielding (>80%) and stereoselective glycosylations were achieved by activation of the thioglycoside donors using *N*-iodosuccinimide in the presence of $La(OTf)_3$[96].

Another report describes a general strategy for the total synthesis of four structurally-related PhGs, echinacoside, acteoside, calceolarioside-A and calceolarioside-B[14]. This strategy features the application of low substrate concentration glycosylation and *N*-formyl morpholine modulated glycosylation methods for the construction of 1,2-trans β- and α-glycosidic bonds. This process consists of 5 synthetic schemes (not shown). Scheme 1 concerns the preparation of thioglucoside building blocks, 4-hydroxyl unprotected thioglucoside and 6-hydroxyl unprotected thioglucoside and the preparation of NAP-protected glucosyl phosphate. Scheme 2 was used to prepare the NAP-protected thiorhamnoside and TBS-protected caffeic acid. Scheme 3 was used to synthesize the phenylethyl disaccharide from building blocks prepared in scheme 1 and phenylethyl alcohol and the echinacoside from building blocks prepared in scheme 2 and phenylethyl disaccharide. Scheme 4 describes the synthesis of the disaccharide intermediate phenylethyl 4-caffeoyl-glucoside 21 for acteoside synthesis and the assembly of acteoside 2. Scheme 5 details the synthesis of calceolarioside-A and the synthesis of calceolarioside-B. The steps in this strategy could not use participatory acyl-protecting groups as these are incompatible with the ester functional group present in target PhG compounds. The antiproliferation properties of these synthetic compounds for the human prostate cancer PC-3 cell line were then studied. The synthesized acteoside was found to exhibit the best antiproliferation capacity.

CONCLUSIONS AND PERSPECTIVES

The various bioactivities of natural products beneficial for human health have attracted scientists to mine the plant kingdom to discover potential PhGs and their functions for clinical drug development. Some of these compounds have been used clinically, *e.g*. acteoside as an antihypertensive drug, and some are still under investigation. The core structure of PhGs comes from phenylpropanoid secondary metabolism. Since the content of these compounds

are limited in their original plants, plant cell and tissue cultures and chemical synthesis have been applied to produce those of high value. Yet, the yield of the cultured cells and tissues has not been sufficient for industrial production and chemical synthesis still required. Synthetic biology may now have the potential to produce valuable natural secondary metabolites.

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