Iridoid glycosides from the Genus *Gentiana* (Gentianaceae) and their Chemotaxonomic Sense

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The genus *Gentiana* is one of the largest genera in the Gentianaceae family. Many *Gentiana* species have been scientifically investigated for their chemical constituents and chemotaxonomic sense. To date, more than 500 chemical compounds were isolated from *Gentiana* plant. Several researchers have reviewed the secondary metabolites and their bioactivities of *Gentiana* plants. However, a survey on iridoid glycosides and their chemotaxonomic sense of the genus *Gentiana* are unavailable. Iridoid glycosides are the major chemicals isolated from *Gentiana* spp., which serve as potential chemotaxonomic markers to differentiate *Gentiana* species. Herein, the review systematically summarizes the scaffolds of the iridoids reported according to the biosynthetic pathway of natural iridoid glycosides. Furthermore, the taxonomy of *Gentiana* and chemotaxonomic sense of iridoid glycosides in the genus *Gentiana* were surveyed.

Keywords: *Gentiana*, Iridoid glycosides, Taxonomy, Chemotaxonomic Sense.

The genus *Gentiana*, comprising almost 400 species, is the largest genus in the family Gentianaceae, with most species growing at high altitudes [1]. *Gentiana* species have been used in traditional folk medicine for a long time. G. lutea, G. asclepiad, G. olivieri, and G. cruciata have been used as antipyretics, stomachics, and stimulants of appetite in Anatolia [2]. G. koehenniana has been used in the traditional medicine in the region of Tuscany [3]. The rhizomes and roots of *G. lutea* and *G. scabra* are the crude drug used as an appetite stimulant in France and Japan, respectively [4, 5]. In the Chinese Pharmacopoeia, the roots of four species from the genus *Gentiana* (Gentianaceae), e.g. *G. scabra*, *G. manschurica*, *G. triflora*, and *G. rigescens*, are used as the raw materials of "Long-Dan" for the treatment of inflammation, hepatitis, rheumatism and cholecystitis [6]. The roots of other four plants from the same genus, e.g. *G. macrophylla*, *G. croatica*, *G. straminea*, and *G. dahuica*, are recorded as the raw materials of "Qin-Jiao" for treatment of jaundice, hepatitis, inflammation, pains and rheumatism [7].

Systematic phytochemical investigation on *Gentiana* has been done since the 1960s. The secondary metabolites from *Gentiana* with not only the promising bioactivities, but also the chemotaxonomic sense have stimulated natural product chemists to study on the genus. To date, more than 500 structurally and biologically diverse compounds have been isolated from the genus, most being iridoids glycosides, flavonoids, xanthones, and triterpenoids, few being lignans and phenolic compounds [7].

A review giving iridoids and their occurrence in *Gentiana* species was reported most 20 years ago [8]. In recent, Y.P. Shi et al described the phychochemical and pharmacological progress of this genus during the period 1960–June, 2009 [9]. Y.Z. Wang et al updated the latest published data of *Gentiana* [7]. However, none of them gave general insight into the iridoid glycosides from the genus *Gentiana* and their chemotaxonomic sense.

As part of ongoing effort to search for the comprehensive compounds from *Gentiana* plants and their chemotaxonomic sense, almost 10 species of the genus *Gentiana* have been studied by our group, which led to the isolation and determination of more than 150 compounds [10–24]. The results stimulated us to systematically review the data that have been reported in the literature from 1960 to date, concerning the taxonomy, iridoid glycosides and their chemotaxonomic sense of the genus *Gentiana*. The names and the corresponding plant sources of all the compounds are compiled in Supplementary Table 1.

**Taxonomy and phylogenetic development of the Gentiana genus**

The taxonomy of *Gentiana* has been studied by most botanists since Linnacus established the genus in 1937 and divided the genus into seven groups [25]. In 1753, Linnacus redivided it into three unnamed sections, describing 23 species [25]. Since then, more than 40 botanists, e.g. Froelich (1796), Grisebach (1845), Kusnezov (1894), Marquand (1928–1937), Smith (1936), Ma (1951), and Ho. (1985), have been published several papers to discuss the infrageneric classification of *Gentiana* [25]. In 1990, Ho. suggested *Gentiana* should be split into 15 sections. Five of them were redivided into several series. As a monophyletic group, *Gentiana* included 23 species and 10 monotypic sections in a systematic revision of all the species of *Gentiana* [25]. The infrageneric classification of *Gentiana* has been accepted by "Flora of China" and approved by most taxonomists, which included 15 groups and 33 series.

However, some taxonomists disagreed about the opinion that Sect. Stenogynye Franchet (Franchet, 1884; Kusnezov, 1894; Pringle, 1978; Ho & Liu, 1990; Yuan & Küppers, 1993) was belonged to *Gentiana* [26, 27]. In 1965, Smith recognized its closer relationship to Tripterospermum and Crawfordia not Gentiana [28]. Löve and Löve (1976) merged it to Tripterospermum [29]. Yuan (1996)
was in agreement with the previous conclusion on the separation it from *Gentiana* based on sequences of internal transcribed spacers (ITS) [1]. Ho. (2002) suggested that Sect. Stenogyne Franchet should be divided from *Gentiana* and erected a new genus in Gentianaceae, named as *Metagentiana* [30]. In 2005, Chen further supported the view based on the analysis of the nucleotide sequence of the ITS of nuclear ribosomal DNA [26].

In addition, Kuznezov (1894) discussed the evolutionary relationships among its infrageneric taxa. Ho. (1996) made an infragenic classification and analyzed the phylogenetic relationships of the infragenic taxa based on the principles and methodes of cladistics [31].

![Chemical structures](image)

**Scheme 1:** The classic mevalonic pathway established leading to the iridoids.

**Iridoid glycosides of the Gentiana genus**

Common iridoids are monoterpeneoids with iridane skeleton. Usually, they are found in a large number of plant families as natural glucosides with a great number of important biological activities such as anti-inflammatory [32], antitumor-chemopreventive [33], and hepatoprotective activities [7].

Herein, we review the presence of iridoid compounds of the genus *Gentiana* (Gentianaceae) and discuss their chemotaxonomic sense. Based on the classic mevalonic acid pathway established leading to the natural iridoids (Scheme 1), the iridoid glycosides reviewed were divided into six groups, e.g. carboxylic iridoids, secoiridoid, wernoside, wernolamins, and gentiopicroside derivatives.

**Carboxylic iridoids**

Carboxylic iridoid was the key intermediate in the biosynthetic pathway of natural iridoid glycosides. To date, more than 40 ones have been isolated from *Gentiana* plants. Among them, the C-7 position of carboxylic iridoids was commonly oxygenated by OH (1-24) [10, 19, 23, 34-44] (Figure 1) and the carbonyl at C-11 hydroxyl at C-7 or glucose unite was acetylated or esterified by aromatic group, e.g. coumaroyl, caffeoyl, feruloyl, 2'-m-hydroxybenzoyl groups. It is note that the structure of depresoside (21) was corrected to 6-β-[2,3-dihydroxyphenyl]-D-glucosyl 7-O (2,3-dihydroxybenzoyl)-loganate [45].

![Chemical structures](image)

**Figure 1:** The structures of carboxylic iridoids.

Moreover, few of them occurred at other positions oxygenated by OH were obtained from the *Gentiana* plants. Gentioside (25) at C-7 and 8 positions oxygenated by OH, were obtained from *G. cruciate* [46] and detected in its clones by HPLC [47]. Ausubin (26) was isolated from *G. kurroo* [48], at C-6 position oxygenated by OH. Harpygoside (27), at C-5, 6 and 8 positions oxygenated by OH, was gotten from *G. macrophylla* [11]. The carboxylic iridoid derivatives oxygenated by OH at C-6, 7 and 8 were obtained from *G. kurroo*, e.g. catalpol (28), 6'-cinnamomoyl catalpol (29), 6-O-cinnamomoyl catalpol (30), 6-O-vanilloyl catalpol (31), and 6-O-feruloyl catalpol (32) (Figure 2) [48]. Three compounds of which C-7 were oxygenated by carbonyl were reported from *Gentiana* genus, e.g. (2,3',6'-tri-O-acetyl-4'-O-trans-p-O-(2,5-di-glucooyranosyl) coumaroyl-7-ketogalamin (33) [43], 7-ketogalamin (34) [43], and 2-(2,3-dihydroxybenzoyl)oxo)-7-ketogalamin (35) [49]. Two compounds not oxygenated at C-7, 6-deoxoyloganic acid (36) and asystasoside A (37), were obtained from *G. apiata* (Figure 2) [50].

Most of carboxylic iridoids were O-glycosides. Non-glycoside iridoids have been rarely reported. Up to now, only two compounds, loganin (38) [51] and stansoside derivative (39) [52] were obtained from *G. verna* and *G. aristata*, respectively. In addition, few rearranged iridoids were identified from the *Gentiana* plants, e.g-
petaside aglucone (40) from *G. aristata* [52], gentianaside (41) on *G. scabra* [42], erythrocentaurin acid (42) from *G. acrophylla* [53], and erythrocentaurin (43) from *G. pneumonanthe* (figure 3) [54].

![Figure 2: The structures of carbocycly iridoids.](image)

![Figure 3: The structure of carbocyclic iridoids.](image)

**Secologanic acid derivatives.**

The bond C7-C8 of carbocyclic iridoids split by free radical reactions to produce secologanic acid derivatives. The derivatives were the key intermediate, which were cycled to get gentiopicroside derivatives or polymerized to produce the bis-iridoid glycosides.

To date, 19 secologanic acid derivatives (Figure 4) were obtained from *Gentiana* plants including few typical secologanic acid derivatives, e.g. secologanoside (44) [19], 6-O-β-glucopyranosyl secologanoside (45) [19] and secoxyloganin (46) [19] from *G. rhodentha*, as well as gentiogeninoside A (47) were obtained from *G. rigescens* [16]. In addition, some secologanol, secologanin and alpigenoside derivatives were isolated from *Gentiana* plants, which were the important intermediate on the classic mevalonic acid pathway of iridoid glucoside biosynthesis. Five secologanol derivatives were secologanol (48), 7,8-acetylsedologanol (49), 7,8-(2,3-dihydroxybenzoyl) secologanol (50) from *G. verna* [55], depressine (51) from *G. depressa* [56], and 6-O-β-glucopyranosyl secologanol (52) from *G. rhodentha* [19]. Secologanin (53) and 7-dioxolanylosecologanin (54) probably being a protected form of secologanin by acetalisation were gotten from *G. verna* [57]. Alpigenoside (55) and 7-demethyl alpigenoside (56) were from *G. rhodentha* [19] and *G. pyrenaica* [58], respectively.

![Figure 4: The structures of secologanic acid derivatives.](image)

![Figure 5: The dimers of secologanic acid derivatives.](image)

In 1994, depresteroside (57) (Figure 5) as the first occurrence of a dimeric iridoid based on secologanoside and loganic acid derivative was obtained from *G. depressa* [59]. In the same year, Liu et al. reported three new similar bis-iridoids (Figure 5), gentiounosides A-C (57–59), from *G. urma* [10][14]. Among them, gentiounoside A and depresteroside (57) have the same structure but different names. Subsequently, the rhodentoside A (60) from *G. rhodentha* [14] was reported, which was the first representative of a new type of bis-secolanic acid linked with a monoterpenne unit from the *Gentianaceae* family. In 1996, two new secologanic acid derivatives, kusnezovides la (61) and lb (62), were isolated from *G. kusnezovii* [15], which was a new tetra-ecologic acid linked with a monoterpenne unit. In the same year, Ma et al. reported two new similar tetrameric secologanic acids, rhodentoside B (being kusnezovides lb) and C (62 and 63) from *G. rhodentha* [13].
Kingsiside derivatives.

Kingsiside derivatives were not the major typical iridoid glycosides in *Gentiana* plants. Up to now, 13 ones were reported from *Gentiana* plants (Figure 6). The derivatives were distributed limilly in *Gentiana* genus, with mainly found in plants of Sect. Stenogyne Franchet. For example, kingside (64) [19], 2′-O-(3′-hydroxybenzoyl)-kingside (65) [19], 8-epikingsiside (67), [19] 6′-O-p-coumaroyl-8-epikingsiside (68), [19] and 2′-O-(3′-hydroxybenzoyl)-8-epikingsiside (69) [19] as the major chemical constituents were isolated from *G. rhodentha*. In addition, 6′-vanilloyl kingside (66) [58] and 6′-vanilloyl 8-epikingsiside (70) were gotten from *G. pyrenaica* [60], as well as 6′-gentsisoyl 8-epikingsiside (71) from *G. siphanantha* [61]. In addition, a few kingside derivatives with a oxygen heterocycles having six-membered rings were from *Gentiana* plants, e.g. morroniside (72), 4′-p-coumaroyl morroniside (73) and 6′-O-[(2R)-methyl-3-veratroyloxypropanoyl]morrionside from *G. pyrenaica* (74) [62], 7(S)-n-butyl morroniside (75) and 7(R)-n-butyl morroniside (76) from *G. straminea* [63, 64].

![Figure 6: The structures of kingsiside derivatives.](image)

Gentiopicroside derivatives.

Gentiopicroside derivatives were not only the major chemical constituents in *Gentiana* genus, but also distributed wildly in it. According to the structure characteristics and biosynthetic pathway of iridoids in *Gentiana* plants, the derivatives were mainly invaded into three types, including gentiopicroside, sweroside and swertiamarin derivatives. Gentiopicroside derivatives were characterized in the existence of double bond in C5 and C-6, while sweroside derivatives without the double bond and swertiamarin derivatives with hydroxyl at C-5.

To date, 25 gentiopicroside derivatives were obtained from *Gentiana* genus, including acylated gentiopicroside in sugar chain (Figure 7), e.g. 3′-acetylgentiopicroside (78) [65] from *G. daharica*, 6′-O-acetylgentiopicroside (79) [63] from *G. straminea*, olivioside C (80) [66] from *G. olivieri*, and olivioside A (86) from *G. olivieri*, as well as the derivatives linked a varying number of saccharide units with their sugar chains, e.g. 4′-O-β-D-glucopyranosylgentiopicroside (81) [67] from *G. scabra*, 6′-O-β-D-glucopyranosylgentiopicroside (82) from *G. asclepiadeou* [68], and scabran G3, G4, and G5 (83-85) [69] from *G. scabra*. In addition, in 2005, gentiassocabrate A (89) [69] obtained from *G. scabra* was one gentiopicroside derivative with hydroxyl and methoxyl groups substituted in C-3 and C-4, respectively. A few of double bond rearranged derivatives, 6β-hydroxywertiapacetasa A (90) [69] from *G. scabra*, wertiapacetasa oxalatoside A (91) [69] from *G. scabra*, 3-cpt-wertiapacetasa C (94) [50] from *G. apiata*, qinjiaoside B (95) from *G. crassicalis* [23], were isolated. It was note that in 1994, qinjiaoside A (90) was isolated firstly from *G. macrophylla* [11], but its configuration was not reported in the literature. In 2005, Masao Kikuchi et al reported it’s relative configuration and named as 6β-hydroxyswertiamacetasa A (90). In 2011, the first novel dimeric gentiopicroside from *Gentiana*, gentinacroside (88) [70], were reported from *G. macrophylla*.

Gentiopicroside derivatives as glycosides commonly existed in *Gentiana* plants. To date, only four non-glycoside gentiopicroside derivatives were reported (Figure 7), e.g. gentiolaviscoside (92) from *G. punctata* [71], (2′)-S-ethylidene-3,4,5,6-tetrahydro-3H-8-dimethoxy-1H,8H-pyrao [3,4-c] pyran-1-one (70) [93] from *G. macrophylla*, as well as new secoiridoid dilactone anantomers (S)- and (R)-gentiolactones (96 and 97) from *G. hosa* [72, 73]. Recently, five new derivatives were isolated from *G. crassicalis* [74], 6′-O-β-D-xylopyranosylgentiopicroside (87) and gentiananosides A-D (98-101). Of them, gentiananoside A (98) and B (99) were concluded to be novel secoiridoid glycosides with an ether linkage between C-20 of the sugar moiety and C-3 of the aglycone. However, the wrong configurations of glucoside moiety of gentiananosides A (98) and B (99) were showed in the publication. Herein, we revised it as Figure 7.

Sweroside derivatives.

Sweroside (102) derivatives (Figure 8) were widely distributed in *Gentiana* genus, especially advanced groups. Few sweroside derivatives linked saccharide units with their sugar chains were gotten and to date, only one, 6′-O-β-D-glucosylsweroside (swertiapunimarin, 103) [75], were reported from *G. macrophylla*.
Iridoid glycosides from the genus Gentiana

Most of them were characterized by the glucose unite acetylated or esterified by various aromatic group, e.g. compounds 104–119 [40, 66, 67, 75–85]. Among of them, trifloroside (107) and sebascoside (108) [79] were strongly bitter acyl sevioside derivatives in Gentiana genus.

In addition, a few sevioside derivatives of which double bond at C-8,10 was hydroxylated or existed a C-8/C-10 oxiranium ring (Figure 9), e.g. 8-hydroxy-10-hydroxysevioside (120) [84] from G. tibetica, and 5-desoxyseviososide (121) [86] G. campestris. Two sevioside derivatives with, 1-O-β-D-glucopyranosylamplexine (122) [82] from G. alpina and 1-O-β-D-glucopyranosyleptomixine (123) [69] from G. scabra, were repoted.

![Figure 8. The structures of sevioside derivatives.](image)

![Figure 9. The structures of sevioside derivatives.](image)

Swertiamarin derivatives.

Similar to sevioside derivatives, few swertiamarin (124) derivatives linked saccharide units with their sugar chains were gotten and to date (Figure 10), only one, e.g. 6'-O-β-D-glucosylswertiamarine (125) from G. alpina [87]. Most swertiamarin derivatives were characterized by the glucose unite acetylated or esterified by various aromatic group (Figure 10), e.g. compounds 126–131 [21, 61, 75, 88–90]. Among them, gelidoside (127, rindoside) [61] was strongly bitter acylsecoeurididic glycosides.

![Figure 10. The structures of swertiamarin derivatives.](image)

Moreover, a few swertiamarin derivatives were from Gentiana genus, of which double bond at C-8,10 was hydroxylated or existed a C-8/C-10 oxiranium ring (Figure 10), e.g. eustoside (132) [91], gentoside (133) [89–90] and eustomumoside (135) [91] from G. gelida, eustoside (134) from G. campestris [86], septemfidoside (136) [92] from G. septemfida. It was note that septemfidoside (136) was iridoid and secoidimar dimer. The ester linkage between an iridoid and sigcoumarid moieties was the common property of all these bis-iridoid compounds, and secoidiods are the acidic unit of all dimers. However, septemfidoside (136) (Figure 10) was the first example in existing swertiamarin moiety and having a carbocyclic iridoid moiety as the acidic part of the ester group. Recently, rigenolide A (137) (Figure 10) was isolated from G. rigescens, which is the first example of structurally unique swertiamarin derivative possessing a cyclobutane skeleton, which was formed by intramolecular [2 + 2] cycloaddition between swertiamarin moiety and p-coumaryl glycoside bond to C-2' of swertiamarin [93].

Chemo taxonomy of the iridoid glycosides.

The aforementioned systematic phytochemical investigation suggested that the carbocyclic iridoids were mainly obtained from Gentiana plants of Sect. Isomeria Kusnez. Phyllocactem T.N.Ho. and Microsporina T.N.Ho. The kingioid derivatives were not the major typical iridoid glycosides in Gentiana plants. They were distributed limitly in Gentiana genus, with mainly found in plants of Sect. Stenoygyna Franchet. The data supported the view of Ho. And Chen that Sect. Stenoygyna Franchet should be divided from Gentiana and erected a new genus in Gentianaceae, named as Metagentiana [26] [30]. Furthermore, from the chemo taxonomy view, the presence of iridoid compounds is characteristic for Gentiana plants. The distribution of iridoids does not correspond to the botanical systematics of plants of them, but at the species level their presence sometimes links species and confirms a phylogenetec relationship.

Conclusion: Most of Gentiana species are important herbs used to treat many diseases by the local people. The iridoid glycosides are the major biological compounds of Gentiana plants. The review survey taxonomy of Gentiana and divided iridoid glycosides into six groups according to their biosynthetic pathway. We also discuss
chemotoxiconomic sense of iridoid glycosides. Furthermore, we pointed a few of same compounds with the different name, especially the iridoid glycosides reported in 1980\textsuperscript{th}. We also revised the compounds showed wrong structures in publication. However, we thought the resolute configuration of the iridoid glycosides reported should be further confirmed.

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