

O-Linked Oligosaccharides in Plant Specialized Metabolites

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[§]SCS-dsm-firmenich Award for the Best Poster Presentation in Chemistry and the Environment

Abstract: Glycosylation is a widespread modification of plant specialized metabolites (PSMs), yet O-linked oligosaccharide chain formation remains poorly explored. Beyond mono-glycosylation, a subset of PSMs occurs as di-, tri-, or higher oligosaccharides conjugates in which multiple sugar units are sequentially attached at a single glycosylation site. Such chain-based architectures have been reported across diverse plant taxa and chemical classes, but remain sparsely documented. Here, we synthesize evidence for O-linked polysaccharides in PSMs, with emphasis on their chemical architectures and biosynthetic principles. Finally, we discuss emerging examples in which O-linked oligosaccharide chains modulate metabolite stability, reversibility, or chemical behaviour, positioning poly-glycosylation as an underappreciated dimension of plant chemical diversity.

Keywords: Chemical ecology · Glycosylation · Plant specialized metabolites · Poly-glycosylation · Secondary metabolism



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1. Introduction

Plant specialized metabolites (PSMs) display remarkable chemical diversity.^[1] Their molecular weight and structural complexity can be expanded by post-synthetic modifications such as glycosylation, influencing solubility, stability, localiza-

tion, and bioactivity. In plants, glycosylation occurs mainly as O-glycosylation, involving labile glycosidic bonds, but also as C-glycosylation, conferring enhanced resistance to hydrolysis. These effects enable plants to accumulate bioactive compounds while limiting their reactivity and cytotoxicity. Beyond mono-glycosylation, PSMs can carry multiple sugars *via* independent additions at distinct positions or through O-linked oligosaccharide chains.^[2] Anthocyanins reflect the structural diversity of poly-glycosylation (Fig. 1). Interestingly, glycosylation can also modulate growth and reproduction.^[3–5] Yet, poly-glycosylated PSMs remain underrepresented in metabolomics and plant biology.

This review synthesizes existing reports on O-linked oligosaccharide chains attached at a single aglycone position, discusses their structural diversity and synthesis, and highlights their functional relevance.

2. Poly-glycosylated PSMs Across Classes

2.1 Aromatic PSMs

2.1.1 Phenylpropanoids

O-linked poly-glycosylated phenylpropanoids have been detected in multiple plant lineages, including spices, crops, fruits, ornamentals, and medicinal plants, suggesting widespread chain-based modification.^[6]

Poly-glycosylated aromatic PSMs frequently display heterogeneous sugar compositions. For example, eugenol O-diglycosides from clove include homogeneous or heterogeneous disaccharides. *Eucalyptus perrinzana* cells convert exogenous eugenol into both eugenyl β -D-glucopyranoside and eugenyl β -gentiobioside.^[7] Expressing the petunia *coniferyl alcohol acetyltransferase* (*PhCFAT*) and *eugenol synthase* (*PhEGS*) in aspen enhanced production of eugenol, eugenol glucoside and eugenol xylose-glucose.^[8] In tomato, *SINSGT1* expression dur-

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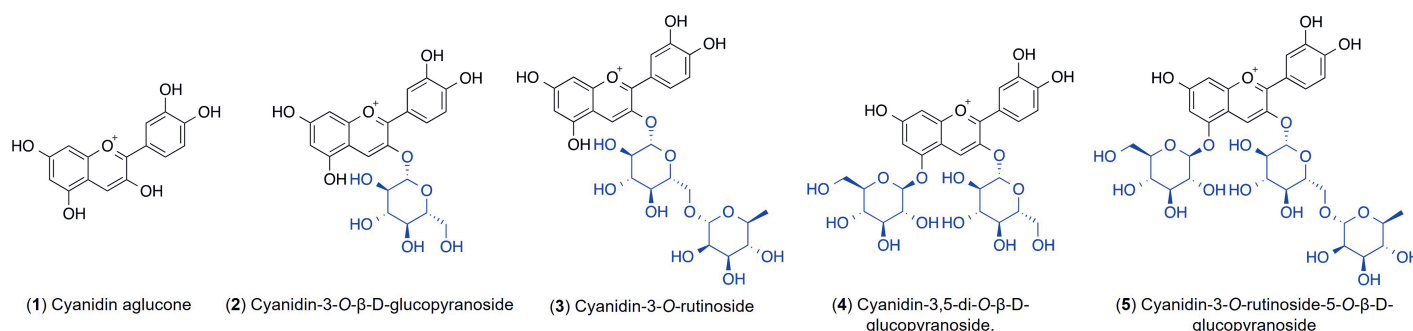


Fig. 1. **Structural diversity of glycosylation architectures illustrated using cyanidin.** (1) Cyanidin (aglycone), the non-glycosylated anthocyanidin core. (2) Cyanidin-3-O- β -D-glucopyranoside, illustrating mono-O-glycosylation at a single position. (3) Cyanidin-3-O-rutinoside [cyanidin-3-O- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside], representing an O-linked oligosaccharide chain attached at a single glycosylation site. (4) Cyanidin-3,5-di-O- β -D-glucopyranoside, illustrating multiple independent O-glycosylations at distinct positions on the aglycone. (5) Cyanidin-3-O-rutinoside-5-O- β -D-glucopyranoside, combining an O-linked oligosaccharide chain with additional O-glycosylation at a second position.

ing fruit ripening converts cleavable eugenol disaccharide into non-cleavable triglycosides.^[5] These studies establish chain-based O-linked poly-hexosylation as a tractable modification of aromatic PSMs.

2.1.2 Flavonoids

Flavonoids are the most extensively documented O-linked poly-glycosylated PSMs. Flavonols, flavones, and anthocyanins frequently occur as di-, tri-, or higher glycosylated derivatives. At the biochemical level, O-linked oligosaccharide chain formation in flavonoids follows a sequential process. In *A. thaliana*, UDP-glycosyltransferases (UGTs) of the UGT78 family predominantly catalyse the initial 3-O-glycosylation of flavonoid aglycones, whereas enzymes from the UGT79 family preferentially act on glycosylated intermediates and mediate chain extension. For instance, glycosylation of kaempferol is first catalysed by flavonol 3-O-glycosyltransferases (GTs) and followed by chain extension with specialized glycoside-modifying UGTs, which converts kaempferol 3-O-glucoside into kaempferol 3-O-sophoroside.^[9]

Beyond disaccharides, flavonols such as kaempferol and quercetin also occur as derivatives bearing gentiatriose, gentiotetraose, and higher homologues.^[10] While the enzymatic steps underlying the initial formation of di-glycosides are well characterized, the specific GTs responsible for the elongation of the oligosaccharide chains remain unresolved. Variation in sugar identity further expands diversity, as seen in kaempferol derivatives such as kaempferol 3-O-arabinoside, kaempferol 3-O-glucoside, and kaempferol 3-O-rutinoside. Together, these examples demonstrate that chain length, sugar composition, and linkage pattern are enzymatically programmed, rather than simple consequences of repeated or unspecific GT activity.

2.2 Nitrogen- and Sulfur-containing PSMs

2.2.1 Benzoxazinoids (BXs)

BXs are major nitrogen-containing PSMs in cereals and are stored as O-glucosides in vacuoles.^[11] These mono-glucosides represent the dominant BX form under steady-state conditions and constitute a canonical example of glycosylation-mediated compartmentalisation in plant defence metabolism.^[11]

Poly-hexosylated BXs have been reported in maize, wheat, and rye, and can be found in cereal-based food products.^[12] Drought stress induced the accumulation of DIMBOA, HMBOA, and HDMBOA, carrying gentiobiose and gentiatriose chains.^[12] Yet, their potential role in responses to abiotic stresses remain unknown.

2.2.2 Cyanogenic glycosides

Cyanogenic glycosides constitute a widespread class of nitrogen-containing plant defence metabolites that release toxic hydrogen cyanide upon tissue disruption.^[13] Examples include linamarin and lotaustralin in cassava, dhurrin in sorghum, and amygdalin in almond and other *Prunus* species.^[13,14] Known cyanogenic diglycosides such as linustatin, neolinustatin, and amygdalin, carry disaccharides such as gentiobiose units directly attached to the aglycone. The addition of these sugar moieties has been reported to be catalysed by two UDP-glycosyltransferases, namely UGT85 and UGT94.^[13]

2.2.3 Glucosinolates (GSLs)

GSLs are a major class of sulfur- and nitrogen-containing plant defence metabolites in which glycosylation is an integral and invariant structural feature. GSLs carry a single thioglucose moiety that is essential for their stability and enzymatic activation by myrosinases.^[15] To the best of our knowledge, no evidence exists for O-linked oligosaccharide chain extension in GSLs.

2.3 Terpenoid-derived PSMs

Terpenoid-derived PSMs, including triterpenoid saponins and steroidal glycoalkaloids, represent one of the most extensively glycosylated classes of natural products in plants.^[16] Like in flavonoids and nitrogen-containing PSMs, glycosylation in terpenoid PSMs involves sequential independent glycosylations of the aglycone scaffold.^[17] In saponins, sugars are commonly attached at defined positions such as the C(3) and/or C(28) hydroxyl groups of the triterpenoid backbone, with each site bearing one or more sugar residues that may themselves form short branches.^[16] Steroidal glycoalkaloids, such as solanine and tomatine, can carry characteristic oligosaccharide moieties (e.g. solatriose, lycotetraose) linked to the steroidal nitrogen-containing core.^[18] As in flavonoids and BXs, these arise from the stepwise extension of a primary glycoside.^[19,20]

3. Biochemical Implications

3.1 Chain-based vs. Scattered Glycosylation Motifs

Chain-based glycosylation concentrates hydrophilicity at one site, generating amphiphilic molecules while preserving aglycone polar surface properties. Such spatial segregation of polar and non-polar regions can influence molecular orientation, interfacial behaviour, and interactions with membranes. In contrast, multi-site glycosylation uniformly masks the aglycone, yielding more isotropically hydrophilic molecules. Chain-based conjugates could, in principle, revert to the aglycone *via* deglycosylation, although reported examples support stepwise terminal

sugar removal. Chain-based glycosylation limits regioisomeric complexity and imposes defined stereochemical organization of sugar residues, which may simplify enzymatic handling and intracellular processing. In contrast, multi-site sugar attachment appears optimized for chemically constrained functions, such as stabilization of reactive intermediates or irreversible masking of functional groups.

3.2 Sequential Assembly and Specificity

O-linked oligosaccharide formation generally follows step-wise sugar addition. In some cases (e.g. eugenol), the same GT catalyses multiple steps, while in others (e.g. flavonoids) specialized GTs mediate chain extension. These enzymes recognize specific aglycone structures, glycosylation positions, and sugar identities.^[2] Evidence from flavonoid pathways demonstrates that enzymes responsible for chain extension preferentially accept mono-glycosylated intermediates, enforcing spatial and architectural control over oligosaccharide formation. In *Petunia hybrida*, UGT79B31 exhibits substrate specificity by acting exclusively on pre-glycosylated flavonoids.^[21] Chain-extending UGTs further differ in their donor sugar preferences, which can influence oligosaccharide composition. For example, UGT79B6 preferentially utilizes UDP-glucose but can also accept UDP-galactose *in vitro*, suggesting that sugar nucleotide availability may modulate chain composition.^[9] The occurrence of dedicated chain-extending GTs indicates that O-linked oligosaccharide formation may result from selection over evolution.

3.3 Chain Length and Sugar Composition

The extent of chain elongation is further shaped by constraints on chain length and sugar composition, which vary markedly among metabolite classes. Flavonoids and BXs tolerate substantial extension of homogeneous glucose chains, giving rise to di-, tri-, and tetra-hexosylated derivatives, whereas cyanogenic glycosides appear to be largely limited to mono- or fixed disaccharide forms. These differences may reflect a combination of steric limitations, enzyme availability, and compatibility with downstream processes.

3.4 Secondary Modifications of Sugar Moieties

In some PSMs, sugar chains serve not only as terminal decorations but also as platforms for secondary chemical modifications.^[22] In anthocyanins sugar chains can undergo further modification, including acylation or additional glycosylation of the sugar residues themselves.^[23] Thus, sugars may constitute pivotal chemical motifs rather than just solubilizing groups.

3.5 Storage vs. Bioactivity

While glycosylation is often associated with metabolite stabilization and storage, the occurrence of O-linked oligosaccharide chains is not uniformly associated with inactivation.^[24] In *A. thaliana*, kaempferol 3-O-neohesperidoside influences auxin transport and homeostasis, ultimately modulating auxin-related developmental processes.^[25] In tomato, kaempferol 3-O-glucosyl-(1→2)-galactoside is required for pollen tube growth.^[26] Additionally, the conversion of cleavable diglycosides of smoky-related phenylpropanoid volatiles into non-cleavable triglycosides prevents their release upon tissue disruption, thereby suppressing aroma formation.^[5] In flowers, the production and accumulation of triglycosides modulates floral coloration.^[3,4] These examples illustrate that O-linked oligosaccharide chains can act as chemical regulators and additional functional studies are required to shed light on their ecological relevance.

4. Outlook and Perspectives

O-linked oligosaccharide chains constitute a chemically distinct and selectively deployed form of glycosylation in PSMs. Key

challenges include distinguishing chain-based from scattered motifs, identifying biosynthetic pathways, and clarifying ecological relevance. Beyond stabilization and storage, chain-based architectures may represent an underexplored layer of PSM biology with potential agricultural applications.

Acknowledgements

We thank dsm-firmenich and the Swiss Chemical Society for the generous poster award. The work was supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (ERC-2019-STG 949595), the Swiss State Secretariat for Education, Research and Innovation (SERI project No. MB22.00052), the Swiss National Science Foundation (Grant No. 310030_189071), and the University of Bern. ChatGPT v5.2 (OpenAI) was used for structuring and wording. The authors declare no conflict of interest.

Author Contributions

All authors wrote the manuscript.

Received: January 20, 2026

- [1] X.-Q. Huang, N. Dudareva, *Curr. Biol.* **2023**, *33*, R473, <https://doi.org/10.1016/j.cub.2023.01.057>.
- [2] T. Louveau, A. Osbourn, *Cold Spring Harbor Perspect. Biol.* 2019, *11*, a034744, <https://doi.org/10.1101/cshperspect.a034744>.
- [3] Y. Morita, A. Hoshino, Y. Kikuchi, H. Okuhara, E. Ono, Y. Tanaka, Y. Fukui, N. Saito, E. Nitasaka, H. Noguchi, S. Iida, *Plant J.* **2005**, *42*, 353, <https://doi.org/10.1111/j.1365-313X.2005.02383.x>.
- [4] S. Sawada, H. Suzuki, F. Ichimaida, M.-A. Yamaguchi, T. Iwashita, Y. Fukui, H. Hemmi, T. Nishino, T. Nakayama, *J. Biol. Chem.* **2005**, *280*, 899, <https://doi.org/10.1074/jbc.M410537200>.
- [5] Y. M. Tikunov, J. Molthoff, de Vos, Ric C H, J. Beekwilder, A. van Houwelingen, van der Hooff, Justin J J, M. Nijenhuis-de Vries, C. W. Labrie, W. Verkerke, H. van de Geest, M. Viquez Zamora, S. Presa, J. L. Rambla, A. Granell, R. D. Hall, A. G. Bovy, *Plant Cell* **2013**, *25*, 3067, <https://doi.org/10.1105/tpc.113.114231>.
- [6] K. Kytidou, M. Artola, H. S. Overkleeft, Aerts, M. F. G. Johannes, *Front. Plant Sci.* **2020**, *11*, 357, <https://doi.org/10.3389/fpls.2020.00357>.
- [7] Y. Orihara, T. Furuya, N. Hashimoto, Y. Deguchi, K. Tokoro, T. Kanisawa, *Phytochemistry* **1992**, *31*, 827, [https://doi.org/10.1016/0031-9422\(92\)80022-7](https://doi.org/10.1016/0031-9422(92)80022-7).
- [8] T. Koeduka, S. Suzuki, Y. Iijima, T. Ohnishi, H. Suzuki, B. Watanabe, D. Shibata, T. Umezawa, E. Pichersky, J. Hiratake, *Biochem. Biophys. Res. Commun.* **2013**, *436*, 73, <https://doi.org/10.1016/j.bbrc.2013.05.060>.
- [9] K. Yonekura-Sakakibara, R. Nakabayashi, S. Sugawara, T. Tohge, T. Ito, M. Koyanagi, M. Kitajima, H. Takayama, K. Saito, *Plant J.* **2014**, *79*, 769, <https://doi.org/10.1111/tpj.12580>.
- [10] T. Tohge, L. P. de Souza, A. R. Fernie, *J. Exp. Bot.* **2017**, *68*, 4013, <https://doi.org/10.1093/jxb/erx177>.
- [11] C. A. M. Robert, P. Mateo, *CHIMIA* **2022**, *76*, 928, <https://doi.org/10.2533/chimia.2022.928>.
- [12] S. Sutour, C. van Doan, P. Mateo, T. Züst, E. R. Hartmann, G. Glauser, C. A. M. Robert, *J. Agric. Food Chem.* **2024**, *72*, 3427, <https://doi.org/10.1021/acs.jafc.3c09141>.
- [13] B. Piršelová, J. Jakubčínová, *Front. Plant Sci.* **2025**, *16*, 1612132, <https://doi.org/10.3389/fpls.2025.1612132>.
- [14] M. Yulvianti, C. Zidorn, *Molecules* **2021**, *26*, 719, <https://doi.org/10.3390/molecules26030719>.
- [15] R. M. Abdel-Massih, E. Debs, L. Othman, J. Attieh, F. M. Cabrerizo, *Front. Microbiol.* **2023**, *14*, 1130208, <https://doi.org/10.3389/fmicb.2023.1130208>.
- [16] N. M. Kavya, L. Adil, P. Senthikumar, *Plant Mol. Biol.* **2021**, *39*, 833, <https://doi.org/10.1007/s11105-021-01293-8>.
- [17] S. Rahimi, J. Kim, I. Mijakovic, K.-H. Jung, G. Choi, S.-C. Kim, Y.-J. Kim, *Biotechnol. Adv.* **2019**, *37*, 107394, <https://doi.org/10.1016/j.biotechadv.2019.04.016>.
- [18] J. Chen, M. Liu, Y. Zhang, F. Bai, *J. Integr. Plant Biol.* **2025**, *00*, 1, <https://doi.org/10.1111/jipb.70077>.
- [19] Y. Liu, X. Liu, Y. Li, Y. Pei, A. Jaleel, M. Ren, *Mol. Hortic.* **2024**, *4*, 43, <https://doi.org/10.1186/s43897-024-00118-y>.
- [20] D.-K. Zhao, Y. Zhao, S.-Y. Chen, E. J. Kennelly, *Nat. Prod. Rep.* **2021**, *38*, 1423, <https://doi.org/10.1039/d1np00001b>.
- [21] E. Knoch, S. Sugawara, T. Mori, R. Nakabayashi, K. Saito, K. Yonekura-Sakakibara, *Planta* **2018**, *247*, 779, <https://doi.org/10.1007/s00425-017-2822-5>.
- [22] S. Wang, S. Alseekh, A. R. Fernie, J. Luo, *Mol. Plant* **2019**, *12*, 899, <https://doi.org/10.1016/j.molp.2019.06.001>.

- [23] Y. Tanaka, N. Sasaki, A. Ohmiya, *Plant J.* **2008**, *54*, 733, <https://doi.org/10.1111/j.1365-313X.2008.03447.x>.
- [24] W. Zhang, L. Chang, Y. Cao, S. Wang, C. Lyu, C. Kang, L. Zhou, I. Huang, L. Guo, *Authorea* **2024**, <https://doi.org/10.22541/au.170665539.90584931/v1>.
- [25] R. Yin, K. Han, W. Heller, A. Albert, P. I. Dobrev, E. Zažímalová, A. R. Schäffner, *New Phytol.* **2014**, *201*, 466, <https://doi.org/10.1111/nph.12558>.
- [26] D. Shin, H. Zhao, E. Tucker, K. H. Cho, D. Liu, Z. Wang, S. Latimer, G. Basset, Y. Wang, Y. Ding, J. Kim, *Plant Physiol.* **2025**, *199*, kiaf556, <https://doi.org/10.1093/plphys/kiaf556>.

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