Plant Polyphenols

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Remarkable Biomimetic Chemoselective Aerobic Oxidation of Flavano-Ellagitannins Found in Oak-Aged Wine**

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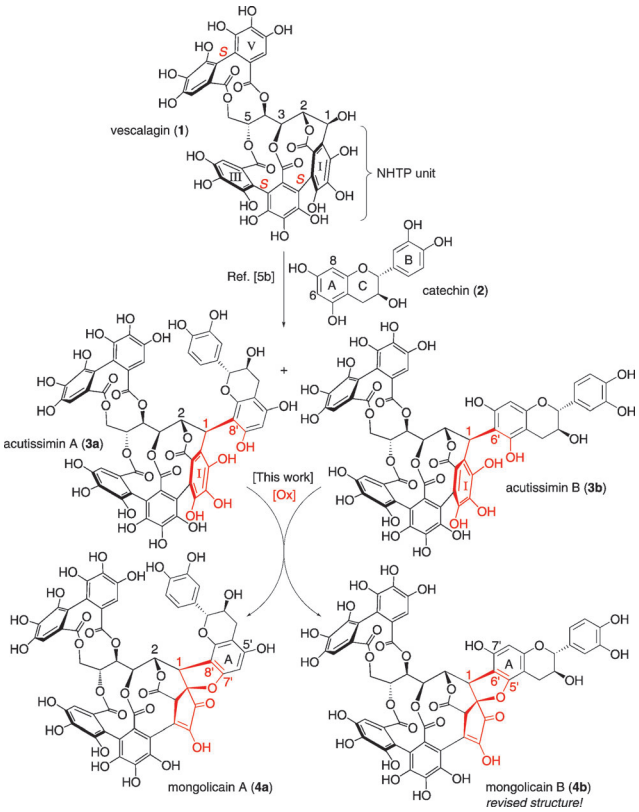
In memory of Yves Glories

Thousands of structurally diverse plant metabolites are represented by the word "polyphenols". Over the last couple of decades, general interest in these structures has been rapidly growing, mainly because of their acclaimed antioxidant properties, which help prevent human disease, and their presence in plant-derived foods and beverages.[1] Among the most characteristic polyphenols are the ellagitannins, a subclass of the so-called hydrolyzable tannins, which are composed of a sugar core, usually D-glucose, to which C–C-linked gallic acid units are esterified. To date, several hundreds of ellagitannins have been isolated from various plant species of the *Angiospermea*, and classified into different groups according to their structural features.[2] One such group is composed of C-glucosidic variants that feature an open-chain glucose connected at its C1 position to their O2 galloyl-derived ring by a C–C bond.[2b,f,3] Vescalagin (**1**)[4] is a representative member of this group of C-glucosidic ellagitannins, in which the C1-linked O2 galloyl ring is part of a terarylic nonahydroxyterphenoyl (NHTP) unit, attached to the O3 and O5 positions of the glucose core through two additional ester bonds (Scheme 1).[2b,f,3]

Our interest in these C-glucosidic ellagitannins was initially sparked by the originality of their structures and by the high selectivity observed when the C1 benzylic alcohol of vescalagin (**1**) takes part in reactions that lead to the diastereoselective formation of numerous derivatives, including natural products. For example, the flavan-3-ol catechin (**2**) efficiently reacts with **1** under mildly acidic conditions to form the regioisomeric acutissimins A and B through nucleophilic substitutions (**3a** and **3b**; Scheme 1).[5] These two compounds



Scheme 1. Formation of mongolicains **4** by chemoselective oxidation of the flavano-ellagitannin acutissimins **3**.

were first isolated from the oak species *Quercus acutissima* (Fagaceae),[6] and were later found to be potent inhibitors of human DNA topoisomerase II.[5,7] Most remarkably, these bioactive flavano-ellagitannin hybrids do occur in wine as a consequence of the ageing of this catechin-containing grape-derived beverage in vescalagin-containing oak barrels.[5,8]

This fascinating contribution of oak-derived C-glucosidic ellagitannins to the chemical profile of wine led us to explore if further chemical transformations of the acutissimins in wine could resemble those of their metabolism in plants. In this context, two known compounds, namely the mongolicains A and B[9] (**4a** and **4b**; Scheme 1), attracted our attention. These two structures feature a spiro-bicyclic dihydrofuran-cyclopentenone unit in place of the bisphenolic moiety of the acutissimins A and B (**3a** and **3b**). Moreover, they are likely obtained from the oxidation of these acutissimins, with which

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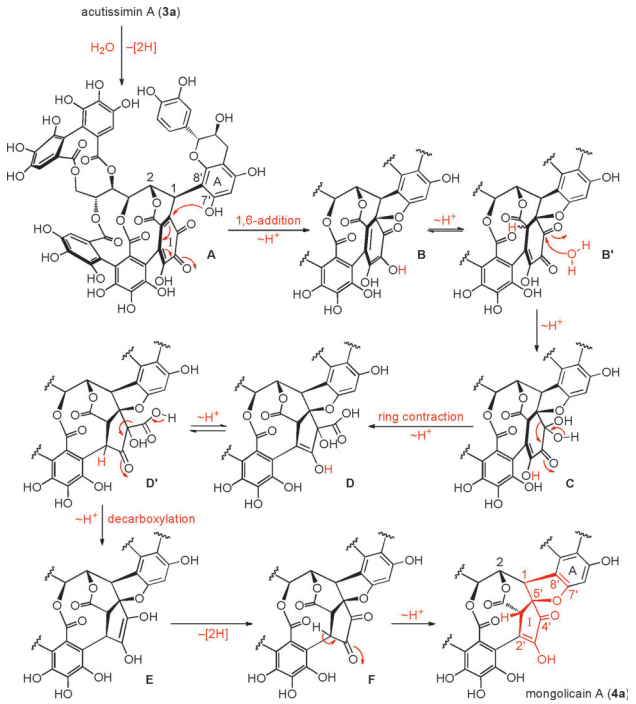
they are known to coexist in the bark of several fagaceous trees.[6,9]

The construction of the spiro-bicyclic dihydrofuran-cyclopentenone unit of these mongolicains occurs through a multistep oxidative transformation, which would start with the dehydrogenation of the C1-linked O2 galloyl ring (ring I) of the vescalagin-derived part of the acutissimins (Scheme 1). As depicted for the formation of **4a** from **3a** in Scheme 2, the



Scheme 2. Mechanism of the conversion of acutissimin A (**3a**) into mongolicain A (**4a**).

resulting electrophilic α-hydroxy-*ortho*-quinone **A** would then be intramolecularly and diastereoselectively trapped by the hydroxy substituent at the 7′ position of the β-oriented ring A of the catechin-derived part of the molecule through a nucleophilic 1,6-addition to deliver intermediate **B**. Prototropic tautomerism would afford the 1,2-diketone **B′**, and water would then attack its more electrophilic carbonyl group. The resulting hydrate **C** would undergo a ring contraction to furnish the cyclopentenol **D**, which could tautomerize into the β-keto carboxylic acid **D′**. Decarboxylation would lead to the enediol **E**, which would need to be dehydrogenated into the 1,2-diketone **F**, before a final tautomerization could lead to mongolicain A (**4a**). Mongolicain B (**4b**) would be similarly obtained from **3b** (for details, see the Supporting Information).

This mechanistic depiction revises and completes those previously proposed in the literature.[3,9,10] The nature of the oxidant that initiates and terminates this sequence of chemical events in planta remains undefined. The intervention of some oxygen-dependent plant polyphenol oxidases (PPOs) that are known to catalyze the oxidation of 1,2-dihydroxyphenyl moieties into *ortho*-quinones can certainly be suspected.[11] PPOs are also present in grapes, but standard fine-wine production practices prevent them from remaining active during the ageing in wooden barrels. However, during this one- to two-year (sometimes even longer) period of wine maturation, oxygen slowly, but constantly, penetrates through the wood and dissolves in the wine solution, in which it is readily consumed as it participates in (aut)oxidation reactions with various wine components, among which phenolic compounds are unarguably first in line.[2b,12] We thus wondered if oxygen alone could promote the conversion of acutissimins (**3**) into mongolicains (**4**). Both acutissimins A and B (**3a** and **3b**)[5] were thus independently dissolved in water and heated at 60 °C in flasks left open to the air. The progress of the reaction was monitored by HPLC analysis; in both cases, the formation of two main products was indicated. For the oxidation of **3a**, the formation of a first major product **5a**, which was clearly observed after about 20 h (not shown), was followed by the gradual formation of a second major product **4a** over the following 50 h; at that time, most of the starting **3a** was consumed (Figure 1). For the oxidation of **3b**, the

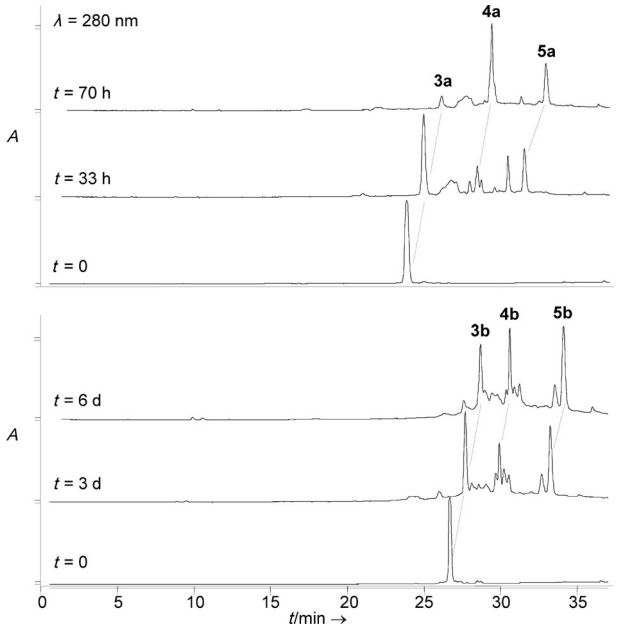


Figure 1. HPLC monitoring of the aerobic oxidation of acutissimins **3** into mongolicains **4** and camelliatannin G analogues **5**.

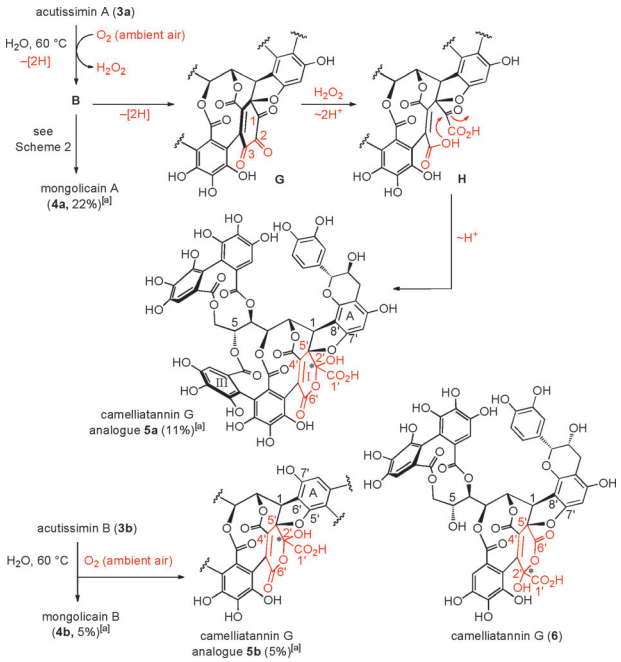
reaction could not be run to completion even after six days, but the two main products (**4b** and **5b**) were present in the final reaction mixture (Figure 1). Electrospray ionization mass-spectrometric (ESI-MS) analysis of the four main products showed signals at $m/z = 1177$ and 1237. We were particularly pleased by the observation of signals at $m/z = 1177$, as they could correspond to the quasi-molecular ions $[M+H]^+$ derived from mongolicains A (**4a**) and B (**4b**).

The four main products were isolated by semi-preparative reverse-phase HPLC. NMR analysis of the two compounds at $m/z = 1177$ confirmed their identification as mongolicains A (**4a**) and B (**4b**). Aside from the ^1H and ^{13}C signals that are typical for flavano-ellagitannins, and similar to those of **3a** and **3b**, striking differences arose in signals for the galloyl-

derived ring I of both **4a** and **4b**. The observation of an α,β-unsaturated ketone resonance at 196.8 ppm ($C4'_I$), and that of an additional proton at around 4.3 ppm ($H1'_I$) were particularly revealing. This proton signal showed HMBC correlations with the four carbon atoms of the cyclopentenone ring located two to three bonds away, as well as with the ester C_I=O and the glucose C1 centers. All 1D and 2D NMR data were consistent with those previously reported by the groups of Nishioka and Kouno for the natural mongolicains A and B (**4a** and **4b**) and related cyclopentenone-containing C-glucosidic ellagitannins. For these, the configurations of the $C1'_I$ and $C5'_I$ centers were established as shown for **4a** in Scheme 2.[9,10,13] However, our interpretation of the NMR data differs from that of Nishioka's group for mongolicain B (**4b**): It is the O5′ substituent, not the O7′ substituent,[9] of the ring A unit of catechin that becomes part of the dihydrofuran moiety of **4b** (see Scheme 1; for details, see the Supporting Information, Figure S16–S19).

The NMR spectra of the two compounds at $m/z = 1237$ (**5a** and **5b**) also displayed ^{1}H and ^{13}C signals typical for flavano-ellagitannins, but the characterization of their galloyl-derived ring I was complicated, owing to the absence of protons on the ring carbon atoms. For both compounds **5a** and **5b**, six carbon resonances were attributed to two carboxyl carbon atoms at 167.7/168.3 ppm and 163.1/163.7 ppm, two oxygenated sp^3 carbon centers at 99.2/97,2 ppm and 86.6 ppm for both **5a** and **5b**, and two doubly substituted olefinic carbon atoms at 130.2/130.0 ppm and 139.3/140.4 ppm. The chemical shifts of these carbon resonances support a structure for ring I that is related to the hemiketalic δ-lactone unit featured in camelliatannin G (**6**), a flavano-ellagitannin isolated from *Camellia japonica* (Theaceae),[10] which does not bear the O5 galloyl-derived ring III featured in **5a** and **5b** (Scheme 3). However, our interpretation of the NMR data, which notably includes a set of diagnostic HMBC correlations between the glucose H1 and $C4'_I$ (139.3/140.4 ppm), $C5'_I$ (86.6 ppm), and $C2'_I$ atoms (99.2/97,2 ppm), leads to a structure for ring I that is a regioisomer of the one proposed for **6**.[10] Under the experimental conditions used, the elaboration of the camelliatannin G analogue **5a** would derive from a dehydrogenation of the ene-1,2-diol intermediate **B** (Scheme 2) to form the triketone **G** (Scheme 3).[14] Its 2,3-diketone unit would then undergo a hydrogen-peroxide-mediated oxidative cleavage[15] to furnish the dicarboxylic acid **H**, which finally leads to **5a**, as depicted in Scheme 3. The camelliatannin G analogue **5b** would be similarly obtained by aerobic oxidation of **3b**. The regioisomeric ring I of **6** would be analogously formed by an oxidative cleavage of the 1,2-diketone unit, but the close correspondence of the ^{13}C chemical shifts for the rings I of **5a**, **5b**, and **6**, and the lack of unambiguous HMBC correlation data for **6**[10] lead us to question the validity of the proposed structure for **6** (for details, see Figures S14, S15, S20, and S21).

The low yield values of isolated **4a**/**5a** and **4b**/**5b** do not reflect their apparent abundance in the HPLC profiles of the reaction mixtures detected by UV-spectroscopy (Scheme 3 and Figure 1) and are due to difficulties in purifying such polyphenolic compounds by semi-preparative reverse-phase HPLC without considerable loss of material (see the Supporting Information for details). Notwithstanding this inefficacy of chromatographic isolation, the aerobic oxidation of **3a**/**3b** into **4a**/**5a** and **4b**/**5b** is a fairly clean and highly chemoselective process. One might then wonder why the dehydrogenative oxidation of the galloyl-derived ring I of **3a** and **3b** is largely preferred over that of the four other pyrogallolic units, as well as one catecholic unit, and one resorcinolic unit. Any of the five pyrogallolic rings of the C-glucosidic ellagitannin parts of **3a** and **3b** should be rather equally prone to oxidation through an initial H-atom transfer, followed by deprotonation into a semiquinone radical anion and one-electron oxidation into an electrophilic α-hydroxy-*ortho*-quinone.[1a,16] However, oxidation of the pyrogallolic ring I leads to the sole α-hydroxy-*ortho*-quinone that can undergo an intramolecular nucleophilic attack from the proximal catechin A ring unit of **3a** and **3b** (for **3a**, see Scheme 2; **A**→**B**). This chemical event would then drive a redox reaction that is otherwise at equilibrium towards the irreversible conversion of **3a** or **3b** into **4a**/**5a** or **4b**/**5b**. The relative efficacy and rapidity of these aerobic conversions, which were strictly performed in the dark using deionized water,[17] is also somewhat remarkable, as the dehydrogenation of catechols/pyrogallols using triplet oxygen is usually an extremely slow (spin-forbidden) process, unless it is activated by transition-metal ions (such as $Fe^{III/II}$ or $Cu^{II/I}$), phenolate-anion-generating bases, or light, or is part of a radical chain autoxidation process.[18,19] The likely intervention of hydrogen peroxide in the diketone oxidative cleavage leading to **5a** or **5b** (Scheme 3) is indicative of an autoxidation process, which could be initiated by traces of iron ions[17] and/or by small amounts of acutissimin-derived phenolate anions.



Scheme 3. Aerobic oxidation of acutissimins **3** into mongolicains **4** and camelliatannin G analogues **5**. [a] Yield of isolated product. * unassigned configuration.

We then performed the aerobic oxidation of **3a** in a weakly acidic standard wine model solution (12 vol% aqueous ethanol, 5 g L^{-1} of tartaric acid, pH 3.2) at ambient temperature, and were gratified to observe the formation of **4a** as the predominant product over the course of 4 months (Figure S2). Finally, we extracted the ellagitannin fraction from a sample of red wine[5b,8] that had been aged for 12 months in an oak barrel, and detected the presence of both mongolicains A (**4a**) and B (**4b**) by LC-ESI-MS analysis (single ion monitoring, positive mode, $m/z = 1177$). Their identification was further supported by comparing their retention times with those of the hemisynthetic compounds (Figure S6).

In conclusion, this work 1) demonstrates that the flavano-ellagitannin acutissimins found in wine undergo aerobic oxidative transformations into mongolicains and camelliatannin G analogues in a remarkably chemoselective fashion for such structurally complex polyphenolic compounds, and hence 2) unveils another new facet of the chemistry of wines aged in oak barrels.

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