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# The Chemistry of Wine Polyphenolic *C*-Glycosidic Ellagitannins Targeting Human Topoisomerase II

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**Abstract:** Polyphenolic nonahydroxyterphenoyl-containing *C*-glycosidic oak ellagitannins are found in wine as a result of the aging of this beverage in oak-made barrels. Once in the slightly acidic wine (pH ~ 3–4), some of these complex natural products such as (−)-vescalagin (**1**), but not its C-1 epimer (−)-castalagin (**2**), can capture grape-derived nucleophilic entities such as ethanol, the flavanols catechin (**10a**) and epicatechin (**10b**), the anthocyanin oenin (**13b**), and the thiolic glutathione (**16**) to furnish condensation products with retention of configuration at the C-1 locus. A computer-aided rationale of this high diastereoselectivity is given. These condensation products can contribute to the modulation of organoleptic properties of the wine, as evidenced by the 23 nm bathochromic shift color absorbance observed with the novel oenin-based anthocyano-ellagitannin (**15b**). Hydrolysis of **1** under solvolytic conditions furnished another novel compound that we refer to as vescalene (**21**), in addition to the known (−)-vescalin (**18**). Of pharmacological importance is the fact that most of these found-in-wine water-soluble ellagitannin derivatives are much more potent than etoposide (VP-16) at inhibiting top2-mediated DNA decatenation in vitro (top2 = topoisomerase II)). The known (−)-vescalin (**18**) and the novel vescalene (**21**) fully inhibited top2 at 10 μM concentration!

**Keywords:** antitumor agents · ellagitannins · natural products · polyphenols · wine

## Introduction

Simple phenols and polyphenols are ubiquitous in fruits, vegetables, and various plant-derived food and beverages that have been claimed to be beneficial for human health.[1] These natural products have also long been regarded as the active principles of numerous plant extracts used in traditional Eastern medicines.[2] Today, the regular intake of fruits and vegetables is highly recommended in the Western diet, mainly because the phenols and polyphenols they contain are thought to play important roles in long-term health and reduction in the risk of chronic and degenerative diseases, such as atherosclerosis and cancer.[3] This increasing recognition of the benefits brought by plant phenols to human health has sparked a new appraisal of various plant-derived food and beverages, such as olive oil, chocolate, apple and citrus juices, coffee, tea, and wine. Their high content in phenolic and polyphenolic substances has recently fuelled numerous investigations that, again, unveiled the therapeutic significance of these natural products, and yet, the potential of polyphenol-based drugs so far has remained untapped in Western conventional medicinal approaches.[3a] The reasons for this relative disapproval of polyphenols by the pharmaceutical industry may be due to the fact that these natural products are usually considered as structurally undefined oligomers only capable of precipitating all kinds of proteins. Hence, standard extraction protocols of plant secondary metabolites usually involve a step to ensure the complete removal of all polyphenolic compounds in order to avoid

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"false-positive" results in screening against specific biomolecular targets.[4] The *C*-glycosidic ellagitannins described herein are examples of biologically active plant polyphenols[5] that unfortunately do not escape this tannin-removing step. These natural products, which are derived from the metabolism of gallic acid,[6] belong to a subclass of highly hydrosoluble ellagitannins[7] that have the particularity, unique among natural products, of featuring an open-chain glucose core. The eight representative natural products displayed in Figure 1 all feature a 4,6-hexahydroxybiphenoyl

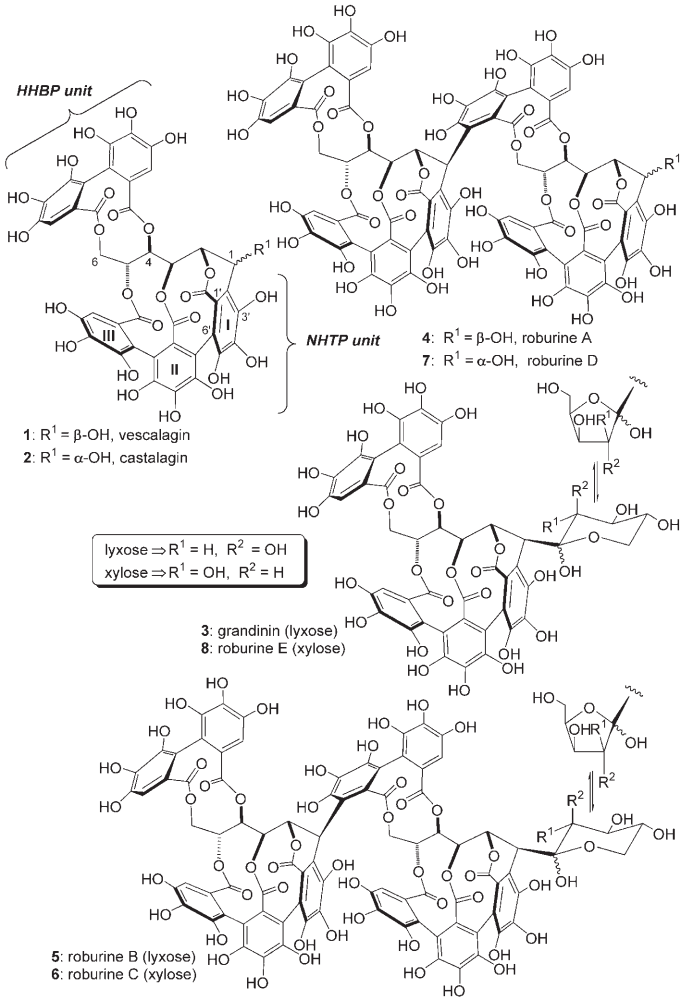



Figure 1. The eight major nonahydroxyterphenoyl (NHTP)-bearing *C*-glycosidic ellagitannins found in *Quercus* and *Castanea* hardwood species.

(HHBP) unit and a 2,3,5-nonahydroxyterphenoyl (NHTP) unit.[8] These pyrogallol-type biaryl and teraryl units are part of eleven- and twelve-membered rings that confer rather rigid and stereochemically well-defined motifs onto these ellagitannins. Their overall globular and preorganized shape makes them potentially better suited for selective recognition of proteins targets[6a,9] than other classes of plant polyphenols, such as the oligoflavanols (i.e., condensed tannins or proanthocyanidins), which adopt helicoidal chain-like structures.[10] Evidence for this better propensity of ellagitannins, with respect to that of oligoflavanols, to interact selectively with proteins can be gleaned from studies on the biological activity of polyphenols and their complexation with proteins.[4a,7b]

The occurrence of NHTP-bearing ellagitannins appears to be limited to plant species from the Fagaceae family of the Fagale order in the Hamameliidae subclass and to species from the families Combretaceae, Lythraceae, Melastomataceae, Myrtaceae of the Myrtale order in the Rosidae subclass.[11] In particular, (−)-vescalagin (**1**) and its C-1 epimer (−)-castalagin (**2**)[12] are found in relatively high amounts in fagaceous hardwoods such as in *Quercus* (oak) and *Castanea* (chestnut) species, in which their content can reach up to 6% by weight of dry heartwood.[13]

The presence of these structurally unique and complex natural products in such high amounts in fagaceous wood species, together with the fact that oak heartwood is the raw material used for the manufacture of barrels in which wine is aged,[14] led us to investigate further their chemical reactivity and their biological activity. We recently reported a preliminary account on the hemisynthesis of topoisomerase-inhibiting flavanoellagitannins starting from **1**.[15] We report herein in full details the results of these investigations on the chemical reactivity of **1** and its epimer **2**, and on the inhibition of human DNA topoisomerase II by these *C*-glycosidic ellagitannins and their derivatives.

## Results and Discussion

The role that *C*-glycosidic ellagitannins play at the molecular level in the elaboration of the chemical profile of wine has been so far mostly overlooked. During aging in oak barrels, the hydroalcoholic and slightly acidic (i.e., pH ~3–4) wine solution enables the solid–liquid extraction of these ellagitannins. Of course, the various long-term seasoning and pyrolytic toasting stages involved in the process of barrel making considerably diminishes the quantity of these compounds available in fresh oak heartwood,[14a,b] but a significant portion of native *C*-glycosidic ellagitannins such as **1** and **2** do resist these drastic conditions.[16] Once in the wine, they are slowly but continuously transformed through condensation, hydrolysis, and oxidation reactions. The premise of our research effort in this field is to elucidate the outcome of these chemical transformations, for their ellagitannin-derived products more than likely contribute to the organoleptic properties of wine.[14c,17] Molecular-level evidence of such a claim are presented in this article focusing on the condensation and hydrolysis chemistry of **1** and **2**.

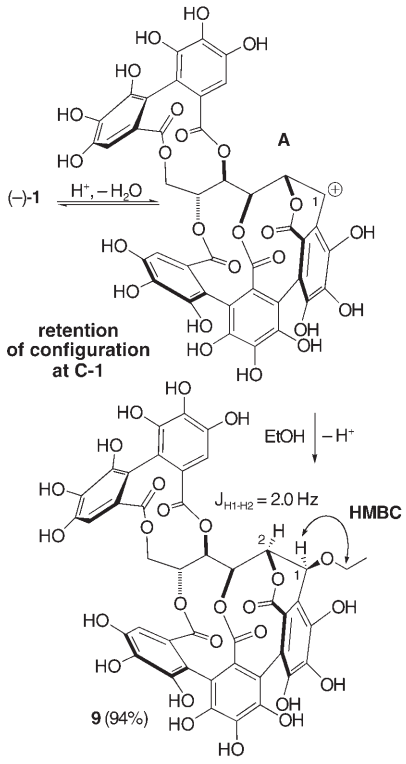
**Vescalagin, a nucleophile "sponge" in wine**: Access to **1** and **2** in significant quantities through standard isolation from oak heartwood[5a,7a] provided us with enough material to undertake a series of condensation reactions. Our experimental approach was based on evaluating first the outcome of the reactions at an analytical scale in an acidic organic solvent system in the presence of some wine-relevant nucleo-

philes. Reaction progress was monitored by high-performance liquid chromatography coupled to electrospray ionization mass spectrometry (HPLC/ESIMS). Products were identified on the basis of their retention time, molecular mass, and mass fragmentation data. Reactions were, in most cases, repeated on a semipreparative scale in the same solvent system in order to obtain the products in sufficient quantities for their full structural characterization by NMR spectroscopy. Some of these reactions were then also carried out in a standard wine model system, consisting of a 12% (v/v) aqueous ethanol solution containing 5 g $L^{-1}$ of tartaric acid at pH 3.2, to verify analytically the formation of their products in such a system.

The first wine-relevant nucleophile we examined was ethanol. Ethanol was added to a solution of **1** in THF containing 1.5% (v/v) of TFA, and the mixture was allowed to react at 60°C for 5 h, after which time a clean conversion into a single product was observed. This product was isolated in 94% yield and its structure was unambiguously determined by mass spectrometry and NMR spectroscopy (see Supporting Information). This vescalagin ethyl ether derivative **9**, the formation of which had never been either observed or even suspected before in wine or wine model solutions (vide infra), results from a straightforward nucleophilic substitution of the vescalagin OH-1 group by ethanol. This connectivity was established from the observation of a strong correlation between the methylenic carbon atom of the ethoxy group and the H-1 proton of the glucose unit in the HMBC NMR spectrum.

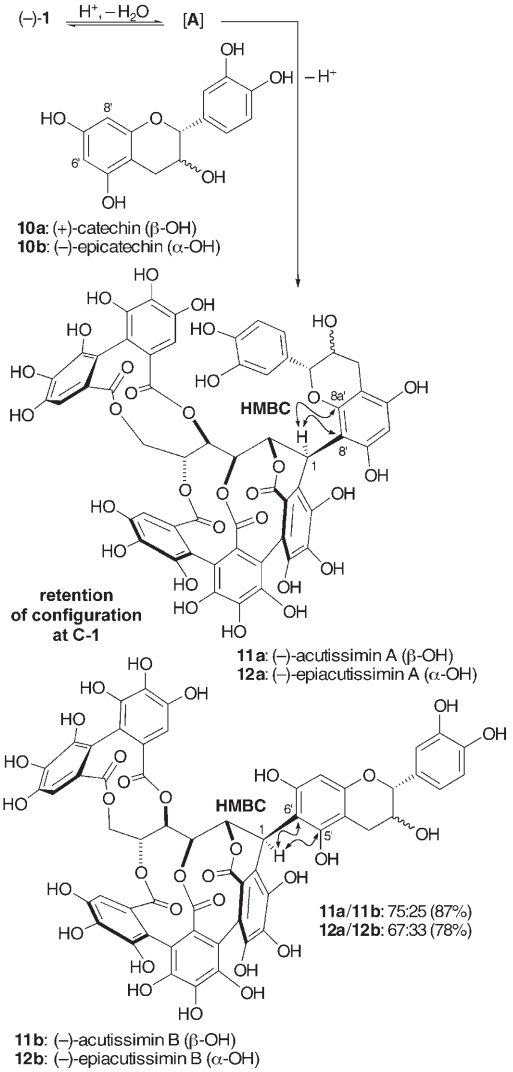
This condensation reaction occurred with full retention of the stereochemistry, since the ethoxy group in **9** is β-oriented at C-1 (Scheme 1). This stereochemistry was deduced from a Karplus interpretation of the small NMR coupling constant between the glucose unit H-1 and H-2 protons; this weak coupling (i.e., $J=2.0$ Hz) indicates that the dihedral angle between these two protons is close to 90° and such an angle is observed when H-1 is α-oriented (Scheme 1).[18] This diastereoselectivity can be considered surprising in view of the $S_N1$-type mechanistic description proposed in Scheme 1.[1b,6b] A computer-aided examination of the benzylic carbocation intermediate **A** actually furnished a clear rationalization of this remarkable stereochemical control (vide infra). This efficient nucleophilic substitution reaction between **1** and ethanol provided us with the motivation for examining other nucleophiles present in wine. At this early stage of our investigation, we became intrigued by the occurrence of acutissimins A (**11a**) and B (**11b**) in the bark of *Quercus* wood species.[18b,19] These flavanoellagitannins are built from a vescalagin- or a castalagin-derived unit connected at C-1 to the C-8′ or C-6′ center of the flavan-3-ol (+)-catechin (**10a**), again in a β-orientation (Scheme 2). The heartwood of *Quercus petraea*, *robur*, and *alba*, the three oak species commonly used to make barrels,[14] does not contain these metabolites, but red wines do contain **10a**, which is derived from grapes, at a mean concentration that has been evaluated to range from about 115 to 190 mg $L^{-1}$).[20] It thus appeared worth examining the possibility of generating acutissimins from oak-extracted **1** and/or **2** and (+)-catechin (**10a**) during wine aging in oak barrels. Answering this question was further stimulated on account of the known potent inhibition of human DNA topoisomerase II (top2) by acutissimin A.[5c] We initially applied the conditions optimized for the preparation of **9** (i.e., 1.5% (v/v) TFA/THF at 60°C) to proceed with the hemisynthesis of both acutissimins from **1** and **10a**. After 7 h, a mixture of both flavanoellagitannins **11a** and **11b** was cleanly obtained and separated by semipreparative HPLC in a 75:25 ratio and 87% yield (Scheme 2). The formation of acutissimin A (**11a**) as the major product is a consequence of the higher nucleophilic character of the more accessible catechin C-8′ center.[21] Since red wines do also contain (−)-epicatechin (**10b**) at a mean concentration of about 80 mg $L^{-1}$,[20] we carried out the same reaction with it to produce the corresponding, but previously unknown flavanoellagitannins, which we called "epiacutissimins" A (**12a**) and B (**12b**), in 78% yield and in a 67:33 ratio, again with retention of the configuration at C-1 (Scheme 2).[15]




Scheme 1. Acid-catalyzed formation of β-1-*O*-ethylvescalagin (**9**) from vescalagin (**1**) and ethanol.

The next step was to verify the formation of these flavanol/ellagitannin hybrids in a reaction system more closely related to wine; this should additionally enable us to confirm the formation of β-1-*O*-ethylvescalagin (**9**). (−)-Vescalagin (**1**) and (+)-catechin (**10a**) were mixed together in the standard wine model solution, and allowed to react at room temperature for a period of 25 days, after which time the two acutissimins A (**11a**) and B (**11b**), as well as ethylvescalagin (**9**), were indeed generated as the major UV-detected




Scheme 2. Acid-catalyzed formation of acutissimins (**11a/b**) and epiacutissimins (**12a/b**) from vescalagin (**1**) and catechin (**10a**) and epicatechin (**10b**), respectively.

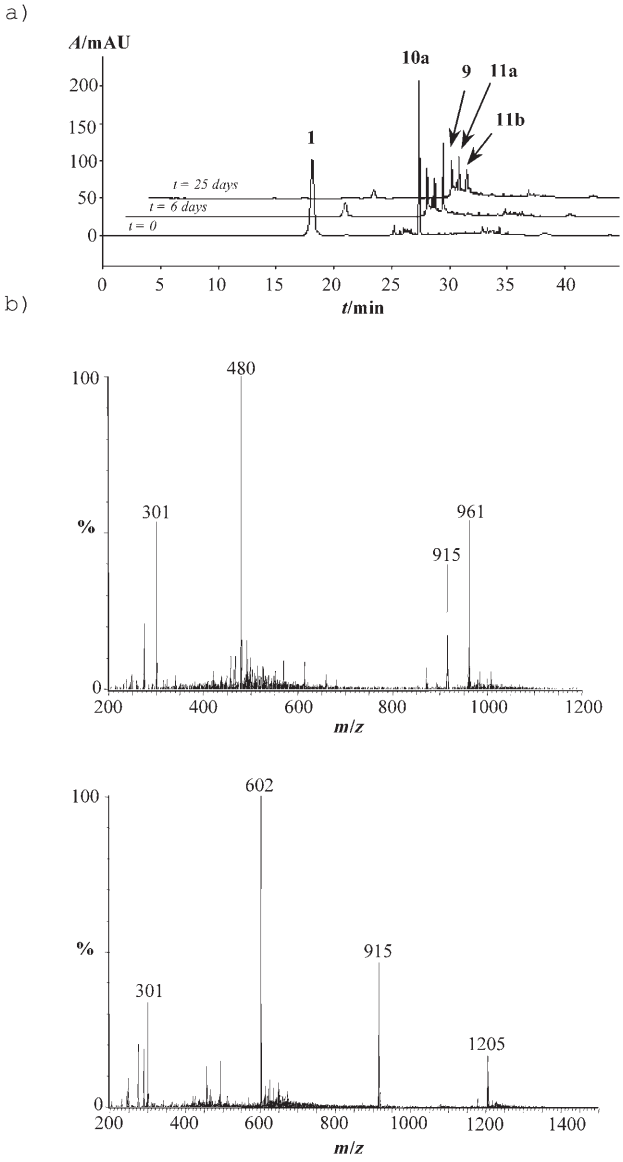



Figure 2. a) HPLC monitoring of the formation of acutissimins A (**11a**) and B (**11b**) and β-1-*O*-ethylvescalagin (**9**) from (–)-vescalagin (**1**) and (–)-catechin (**10a**) in the wine model solution. b) Negative mode (–60 eV) ESI mass spectra of β-1-*O*-ethylvescalagin (**9**, top) and acutissimins A or B (**11a**/**11b**, bottom).

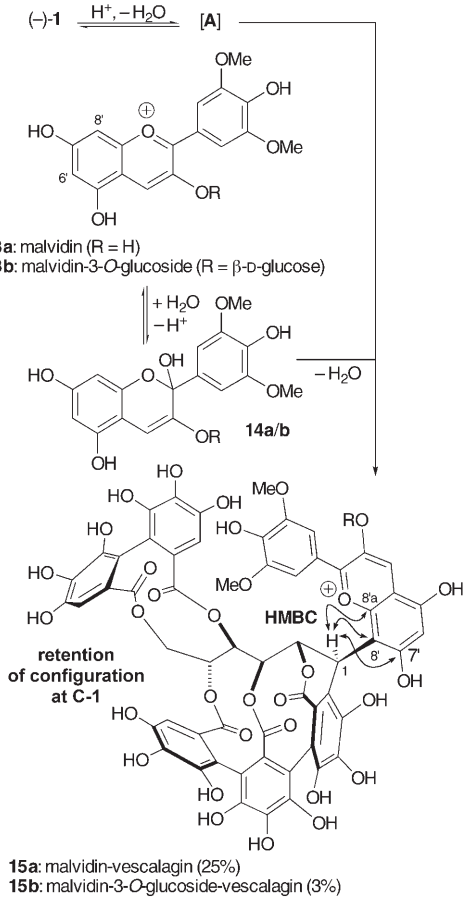
products (Figure 2). It remained to provide evidence of the presence of the acutissimins in wine, which we did by analyzing a sample of red wine that had been aged for 18 months in oak barrels. Not only were the two acutissimins A and B (**11a/b**) detected, but also the two new "epiacutissimins" A and B (**12a/b**). A HPLC/ESIMS-based quantitative determination of their occurrence in the same sample indicated content values of 0.4 mg $L^{-1}$ for **11a**, 0.28 mg $L^{-1}$ for **11b**, 0.30 mg $L^{-1}$ for **12a**, and 0.35 mg $L^{-1}$ for **12b**.[22] Although they appear to constitute relatively minor components in wine, their occurrence is another proof of the participation of oak *C*-glycosidic ellagitannins in the elaboration of the chemical profile of wine. Furthermore, we would like to emphasize here that any quantitative analysis by any available method of any compound at any given time in an aging wine is rather pointless, for wine is a complex multicomponent reaction system that slowly but continuously evolves under mildly acidic and oxidative conditions. As far as the flavano-ellagitannins **11a/b** and **12a/b** are concerned, they are certainly further transformed in wine, but they will continue to form as long as the flavan-3-ols **10a/b** and the *C*-glycosidic ellagitannin **1** are present in the medium. Of relevant note is the detection of both (–)-vescalagin (**1**) and its epimer **2** at concentrations of 2 mg $L^{-1}$ and 8 mg $L^{-1}$, respectively, in the wine sample we analyzed. Other analyses have indicated amounts comprised between 0 and 7 mg $L^{-1}$ for **1** and between 5 and 21 mg $L^{-1}$ for **2**.[16a,b] The fact that **2** is always found in higher amounts than **1** can be explained by their difference in chemical reactivity, as we shall discuss below.

The efficient hemisynthesis of the flavano-ellagitannins **11a/b** and **12a/b** from **1** and **10** in an acidic organic solution

and the proof of their formation in wine then led us to contemplate yet another similar condensation reaction between **1** and a grape-derived flavanoid anthocyanin pigment. Color is an important organoleptic factor in the technical tasting and quality of wine. Numerous investigations have been dedicated over the years to the understanding of the physicochemical mechanisms that underlie red wine color modulation during aging and conservation. Most of these studies evidenced 1) physical co-pigmentation phenomena resulting from stacking of the colored anthocyanin flavylium cations with other wine phenolic species,[23] 2) complexation with metallic cations,[24] and 3) chemical reactions between the anthocyanins and either nucleophilic or electrophilic other wine species (e.g., flavanols, ethanal, and pyruvic acid) that produce new pigments with different coloring properties.[25] Surprisingly, again, none of these studies considered the contribution of *C*-glycosidic ellagitannins to covalent modifications of grape anthocyanins.

To re-address this question of paramount importance for the influence of aging of wine in oak barrels on its quality, (−)-vescalagin (**1**) and the anthocyanidin malvidin (**13a**), the aglycone of the major grape 3-*O*-glucosidic anthocyanin oenin (**13b**), were dissolved in 1.5% (v/v) TFA/THF and allowed to react at 60°C for 24 h. A clean formation of the expected condensation product **15a** was observed (see Supporting Information). This new anthocyanoellagitannin was isolated in only 25% yield (Scheme 3), owing to some unavoidable transformations during its purification by semipreparative reverse-phase HPLC, eluting with acidic water/methanol-based solvents (vide infra). Nevertheless, this reaction constitutes another example of the participation of **1** in substitution reactions with wine-relevant nucleophiles. The connectivity between the vescalagin- and the malvidin-derived units was established from observation of diagnostic two- and three-bond correlations between H-1 and C-8′, C-8′a, and C-7′ in the HMBC NMR spectrum (Scheme 3). Retention of configuration at C-1 was again simply deduced from the small coupling constant observed between the glucose H-1 and H-2 protons. Most importantly for the sake of the color modulation of red wine, the visible spectrum of **15a** revealed an absorbance maximum at 545 nm that is bathochromically shifted from that of malvidin (**13a**) at 517 nm (see Supporting Information). Having thus premièred the formation of an anthocyanoellagitannin, we then examined the same reaction using oenin (**13b**), which is present in red wines in amounts ranging from approximately 24 to 240 mg $L^{-1}$,[20b,26] both in the TFA/THF medium and in the wine model solution. The presence of additional nucleophilic alcoholic functions on **13b** was expected to render the reaction system much more complex than in the case of **13a**. We were thus very pleased to observe, among several other species, the formation of the desired condensation product **15b** after three days at 60°C in the acidic organic solution. Under these conditions, the glycosidic bond of **13b** did not resist cleavage as evidenced by the detection of **13a** and **15a**. Despite the difficulties we again encountered to separate this complex reaction mixture by semipreparative




Scheme 3. Acid-catalyzed formation of the malvidin/vescalagin and malvidin-3-*O*-glucoside/vescalagin condensation products **15a** and **15b** (isolated yield).

HPLC, we managed to isolate **15b** in 3% yield (Scheme 3). Several runs of this reaction provided us with enough purified **15b** to confirm its structure by NMR spectroscopy. Its configuration at C-1 and the connectivity between the ellagitannin and the anthocyanin units were established as for **15a** (Scheme 3). We were then further gratified by the evidence of the formation of **15b**, after letting **1** and **13b** react at room temperature for four months in the wine model solution at pH 3.2 (see Supporting Information).

It must now be recalled that anthocyanins adopt different structures in aqueous solutions according to their pH value. At pH 3.2, the red-colored flavylium form of an anthocyanin, such as oenin (**13b**), typically constitutes only about 20% of its global amount.[25d,27] The most abundant form (~40%) under which anthocyanins exist at this pH value is their colorless hemiacetal form or carbinol base, such as **14b**, which results from the addition of water onto the C-2′ center of **13b** (Scheme 3). The other forms are bluish quinone methides (~15%) and pale yellow chalcones (~25%, not shown).[27,28] This multicomponent system thus exposes several co-existing anthocyanin-derived species in the wine model solution, all of which are capable of reacting with **1**. Furthermore, this pH-dependent equilibrium between the aforementioned anthocyanin forms is evidently still opera-

tional once an anthocyanin unit has been condensed with **1**, hence multiplying the number of conceivable products of this reaction. This situation emphasizes the importance of having first hemisynthesized **15b** in an organic solution, for without this new anthocyanoellagitannin pigment to hand, we would not have been able to unambiguously confirm its formation in the wine model solution.

As for **15a**, the visible absorption band of **15b** is bathochromically shifted by more than 20 nm with respect to that of the anthocyanin **13b**, thus turning the bright red color of **13b** into a deeper red-purple color (Figure 3), which is in

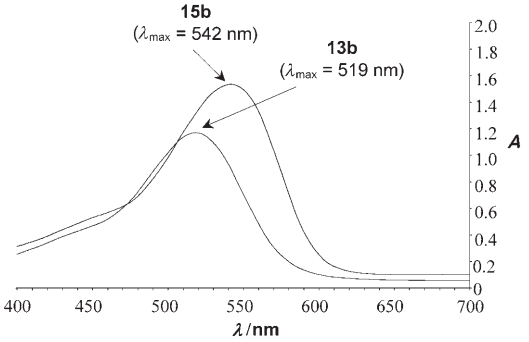
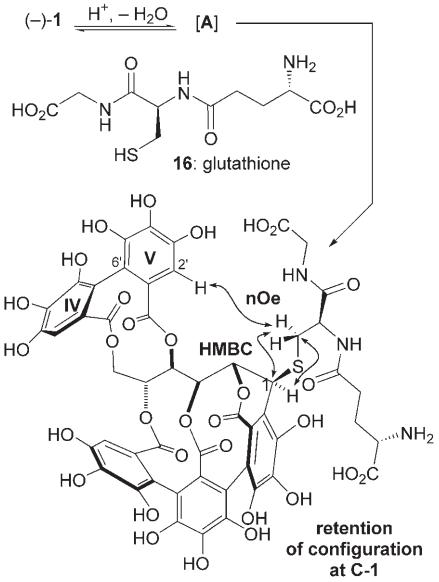



Figure 3. Visible spectra of malvidine-3-*O*-glucoside (**13b**) and malvidine-3-*O*-glucoside/vescalagin (**15b**) in 0.1 mM aqueous solutions at pH 1 and 25 °C.

agreement with the purple tints observed in young red wines. These visible spectra were obtained from aqueous solutions of **13b** and **15b** at pH 1 to ensure that these anthocyanins were entirely in their flavylium forms. The mechanistic description of the formation of **15a/b** proposed in Scheme 3 involves passage by the carbinol bases **14a/b**. Indeed, the positively charged flavylium ions of **13a/b** may suffer from electrostatic repulsion in their approach toward the vescalagin-derived benzylic cation **A**. It is generally accepted that flavylium ions are poor nucleophiles and that their participation in nucleophilic addition processes in aqueous media implies preliminary hydration into the hemiacetal forms **14**, which are more prone to express their nucleophilicity at their C-8′ center.[29] Although we performed the hemisynthesis of **15a/b** in anhydrous THF, the participation of the equivalent of water released from the acid-catalyzed generation of **A** is here implied in the generation of the intermediate **14a/b** (Scheme 3).

The last wine-relevant nucleophile we investigated in this study was glutathione (**16**). This cysteine-containing tripeptide is present in wine musts at concentration ranging from 3 to 24 mg $L^{-1}$ and protects volatile thiols, such as 4-mercapto-4-methylpentan-2-one and 3-mercaptohexanol that contribute to the fruity aroma of white wines, against oxidative degradation. Interestingly, aging of these wines in new oak barrels considerably diminishes the level of glutathione (**16**).[30] It is known that **16** can engage its thiol function in nucleophilic addition reactions with orthoquinones derived from oxidized must caffeoyl tartaric acid,[31] but the aforementioned enologic observations call for another chemical explanation of its disappearance in oak-aged wines. We thus examined the capability of **16** to engage its thiol group in the same condensation reaction as that followed by the other wine nucleophiles previously used in this study. After 34 days in the 1.5% (v/v) TFA/THF medium in the presence of an equimolar amount of **1**, compound **16** was converted into the expected 1-*S*-glutathionyl derivative **17a**, which was isolated in 51% yield (Scheme 4). Connectivity signals con-




Scheme 4. Acid-catalyzed formation of β-1-*S*-glutathionyl vescalagin (**17a**) from vescalagin (**1**) and glutathione (**16**) (isolated yield).

sistent with this condensation product were observed in the HMBC NMR spectrum, notably three-bond through-sulfur correlations between the glutathionyl $-CH_2S-$ and the *C*-glycosidic CH-1 centers (Scheme 4). The configuration at C-1 was as usual deduced from the small coupling constant between the glucose unit H-1 and H-2 protons, here further confirmed by the observation of noe signals between the two $-CH_2S-$ protons and the galloyl-V H-2′ proton. The same reaction carried out in the wine model solution also evidenced the formation of **17a**, together with that of its sulfoxide form (**17b**) and, again, that of β-1-*O*-ethylvescalagin (**9**) (see Supporting Information).

**Why not castalagin?** All the nucleophilic substitution reactions described above have been performed with (−)-vescalagin (**1**). We had also attempted to use its epimer (−)-castalagin (**2**) for generating the acutissimins **11a/b**, but this attempt was to no avail. In fact, in their first trial to hemisynthesize **11a** from **10a** and **2** in anhydrous dioxane containing *p*-toluenesulfonic acid, Ishimaru and co-workers only produced small amounts (i.e., 3.7%) of the desired flavanoellagitannin.[18b] This refractory behavior of **2** has been previously documented,[5a,13c,18a,32] but it remains nevertheless striking when one considers that the only structural difference between these two epimers of relatively high molecular weight (i.e., 934 Da) is the orientation of the OH group at C-1.

Strain energy calculations we performed indicated that **2** is more stable than **1** by only 2.9 kJmol$^{-1}$.[5a] The corresponding minimum-energy conformations that were identified by using the MM3* force field showed that the β-oriented OH group of **1** is directed outward from the less crowded face of the molecule, whereas the α-oriented OH group of **2** is embedded in the structure and predisposed to participate in an intramolecular O-1···HOC-3′ H-bond of 2.21 Å at an angle of 146° (Figure 4a and b). The involvement of the O-1 atom in this H-bond would then lower its basicity, hence rendering **2** reluctant to protonation at this site. Furthermore, departure of a protonated OH-1 group may be favored from **1**, since this OH group is positioned in a more energetically demanding pseudo-axial orientation on a six-membered lactone ring, whereas it is in a pseudo-equatorial orientation in **2** (Figure 4c).[1b] Together, these stereoelectronic arguments constitute the best rationale we can propose to explain the quasi total inertness of **2** as compared to the reactivity of **1** under the same reaction conditions.

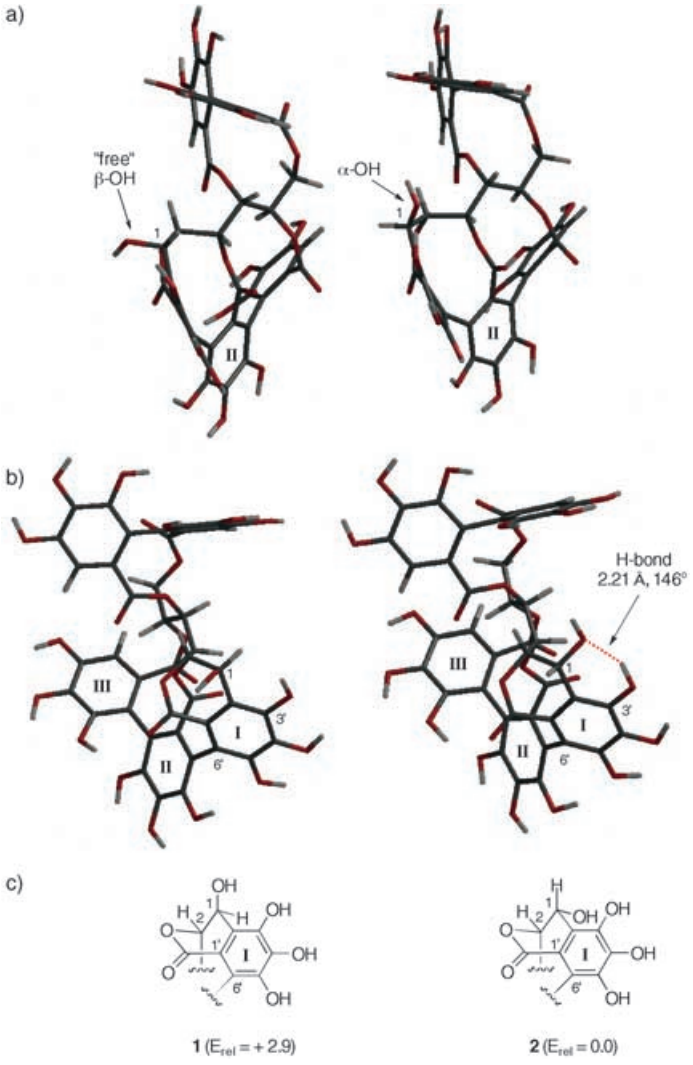



Figure 4. MM3* minimum-energy conformations and relative strain energies (kJmol$^{-1}$) of vescalagin (**1**) and castalagin (**2**). a) back-face views; b) front-face views; c) depictions of the OH-1 group orientation on the six-membered ring lactone motifs of **1** and **2**.

**Diastereoselective control**: The other remarkable aspect of the chemistry of (−)-vescalagin (**1**) is that all the nucleophilic substitution reactions described herein occurs with full retention of configuration at C-1. In fact, all known complex *C*-glycosidic ellagitannins bear their C-1 carbon-based substituent in a β-orientation. Our results lay down the evidence that this stereochemical preference is not under enzymatic control during the biogenesis of these plant metabolites. As alluded to above, this may appear somewhat surprising when one invokes passage by the intermediate carbocation **A** to describe these reactions.[1b,6b] We thus calculated the lowest-energy unoccupied molecular orbital (LUMO) of **A** using a Spartan-generated Hartree–Fock model (Figure 5a). A large and symmetrical sp$^2$-hybridized atomic orbital is expectedly located at C-1. The only argument for a preferred attack of a nucleophile from the *exo* β-face of this orbital would be that its α-oriented lobe is less accessible for bond formation because of steric impediment on the *endo* face of the molecule. However, another inter-

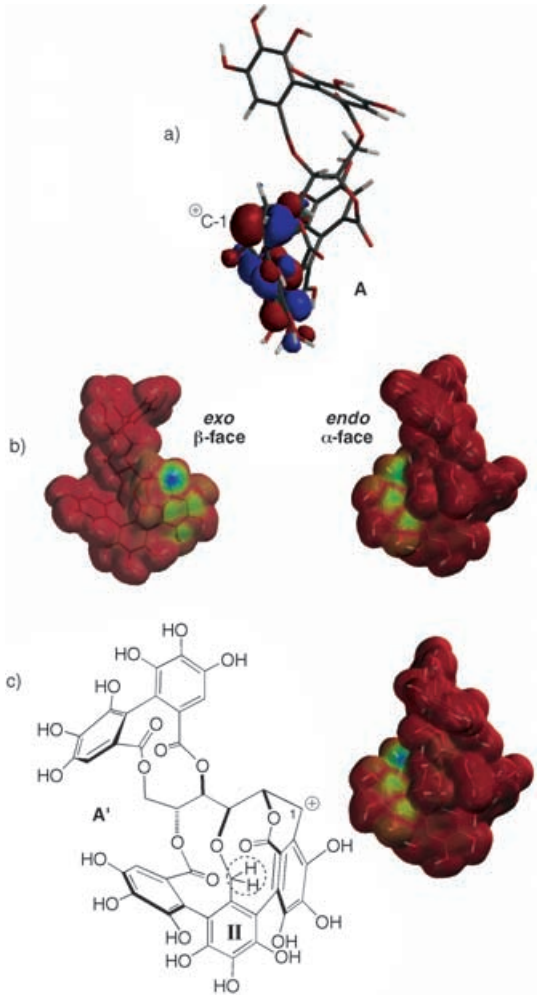


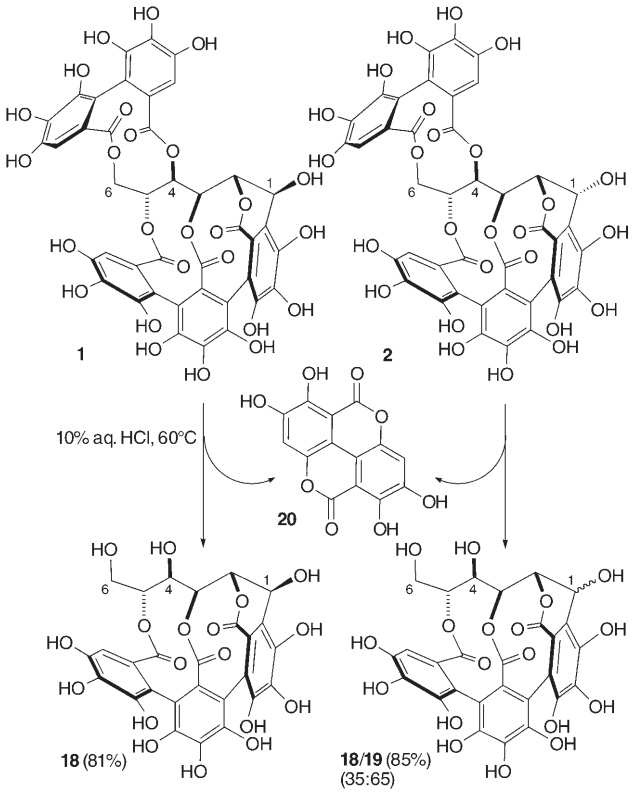

Figure 5. a) Spartan-generated Hartree-Fock model of the LUMO of the vescalagin (**1**)-derived benzylic cation intermediate **A**. b) Mapping of the same LUMO onto the 0.002 electron au$^{-3}$ electron density isosurface of **A**. c) Same mapping of the LUMO of the benzylic cation **A′** for which the galloyl-II carbonyl unit of **A** has been replaced by a methylene unit. The bluer the color, the more electron deficient the orbital is.

esting issue was revealed when mapping this Hartree–Fock LUMO model onto the 0.002 e au$^{-3}$ electron density isosurface of **A** (Figure 5b).[15] These color-coded displays indicate that the *exo* face of the orbital is more electronically-deficient (deeper blue color) than its more encumbered *endo* face. This suggests that this orbital might be under the electronic influence of an adequately oriented neighboring electron-rich group or atom. A closer examination of the structure of **A** identified the carbonyl oxygen atom of the galloyl group II of the NHTP unit as appropriately disposed to participate in such an orbital interaction. This carbonyl group was thus replaced in silico by a methylene unit and mapping of the LUMO of the resulting cation **A′** on its electron density isosurface showed significant recovery of the electron-deficient nature of the *endo* α-face of the C-1 orbital (Figure 5c). Hence, this trivial computer-based inspection of the benzylic cations **A** and **A′** provides a meaningful illustration of the stereoelectronic factors that control the diastereofacial differentiation observed in the nucleophilic substitution chemistry of vescalagin (**1**).

**Hydrolysis of vescalagin and castalagin**: In addition to the eight major NHTP-bearing *C*-glycosidic ellagitannins represented in Figure 1, oak heartwood contains two other members of this class of polyphenols. These two compounds, named (−)-vescalin (**18**) and (+)-castalin (**19**),[12c] are also relevant to the present investigation, since they are also extracted from oak by the wine during aging.[16b] Their occurrence in oak presumably results from hydrolytic cleavage of the 4,6-HHBP unit of **1** and **2**, and their presence in wine can additionally be due to the same hydrolysis taking place in the wine itself. We thus independently submitted **1** and **2** to hydrolysis under acidic conditions adapted from the method previously described by Scalbert and co-workers.[14e,33] After 39 h at 60 °C in a 10 % aqueous HCl solution, (−)-vescalagin (**1**) was converted into **18** in 81 % yield with concomitant formation of ellagic acid (**20**), the bis-lactone formed from the release of the 4,6-HHBP unit (Scheme 5).

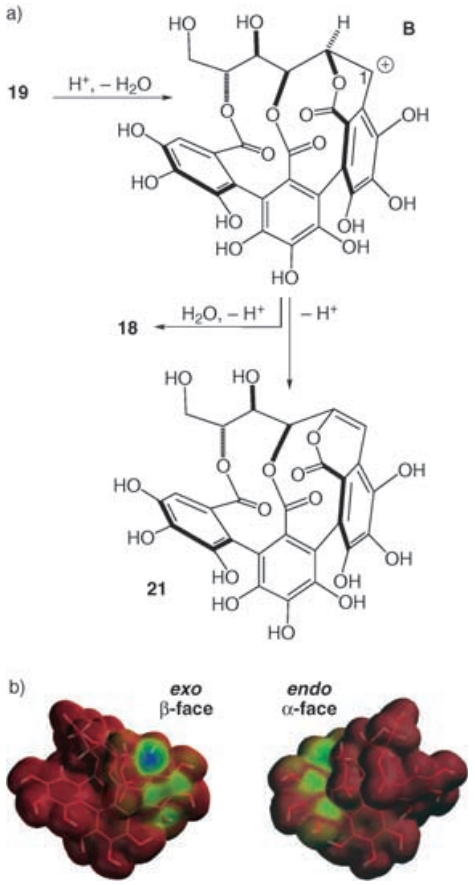
Hydrolysis of (−)-castalagin (**2**) took longer (i.e., 65 h) to go to completion under the same conditions, and surprisingly led to a 65:35 mixture of **19** and **18** in 85 % yield (Scheme 5). Other minor products of unknown structures were detected, but no attempt was carried out to isolate them at this stage (vide infra). Since no formation of **1** was detected during HPLC monitoring of the hydrolysis of **2**, it would seem that the formation of **18** was due, in this case, to an epimerization of **19**. Water would thus be capable, under the solvolytic conditions used, of displacing the OH-1 group of **19**, but not that of **2**. If one assumes a stepwise process with passage by a benzylic cation **B**, this intermediate would then be exclusively trapped by water from its *exo* β-face to furnish **18** (Scheme 6). Admittedly, this description fits nicely with the behavior of the vescalagin-derived benzylic cation **A**, which also cannot undergo nucleophilic attack from its α-face (vide supra), but does not explain why **19** can epimerize into **18** and not **2** into **1**. The fact that **19** has an α-face that is much less encumbered than that of **2** may




Scheme 5. Preparation of vescalin (**18**) and castalin (**19**) by acid hydrolysis of vescalagin (**1**) and castalagin (**2**) (isolated yields).

facilitate the displacement of its protonated α-OH-1 group, perhaps further helped by some incoming water from the β-face in a concerted manner. Once **18** is thus formed, a stable benzylic cation intermediate (**B**) derived from it would have the possibility of leading to other minor products, in addition to giving back **18**, but not to **19**. The chemistry of **B** would hence not be strictly governed by steric factors, but mainly by the electronic distribution onto its LUMO, the *endo*-face of which being under the same stabilizing stereoelectronic influence as that of **A** (Figure 5). As expected from this hypothesis, the Spartan-generated model of the LUMO mapped onto the electron density isosurface of **B** again showed that the *exo*-face of the orbital centered at C-1 is clearly more electron-deficient than the *endo*-face (Scheme 6). In order to confirm the epimerization event, the vescalin/castalin (**18**/**19**) mixture was separated by semi-preparative HPLC, and the resulting pure **19** was resubmitted to the same solvolytic reaction conditions. After six days, **19** was completely converted into two major products, which were separated to furnish pure vescalin (**18**) in 65 % yield, and another new product in 21 % yield, which turned out to be the major secondary products observed during the hydrolysis of **1** and **2** (Scheme 6). The structure of this compound, which we refer to as vescalene (**21**), was unambiguously determined by NMR spectroscopy and mass spectrometry (see Supporting Information). The formation of **21** is thus a consequence of the engagement of **B** into an $E_1$-type elimination process. The possibility of a concerted $E_2$-type




Scheme 6. a) Acid-mediated epimerization of castalin (**19**) into vescalin (**18**) and elimination into vescalene (**21**). b) Spartan-generated Hartree–Fock model of the LUMO of benzylic cation **B** mapped onto its 0.002 electron $au^{-3}$ electron density isosurface.

elimination from **18** is here dismissed on the basis that the other two primary and secondary alcoholic functions of the glucose moiety do not engage in any elimination reaction. This fact lends further credit in favor of an exclusive passage by a highly stabilized secondary benzylic carbocation **B** (Scheme 6).

**Inhibition of topoisomerase II mediated decatenation in vitro**: A few studies have addressed the biological activity of NHTP-containing *C*-glycosidic ellagitannins. We have already shown that **1**, **2**, **3**, **5**, and **7** (Figure 1) selectively inhibited the replication of acyclovir-resistant herpes simplex strains of type 1 and 2; (−)-vescalagin (**1**) is extremely active and exhibits antiviral activity at subfemtomolar concentrations with a selectivity index $5\times10^5$ times higher than that of acyclovir.[5a] Selective antiproliferative activity have been reported in melanoma cell lines for **1**, **2**, **3**, and **11a** with $ED_{50}$ values ranging from 0.1 to 1 μM.[5d] A 1-*O*-galloylated derivative of **2** was also shown to induce apoptosis in human leukemia cells with an $ED_{50}$ of about 11 μM.[34] Another study reported that **11b** exhibits a strong gastroprotective effect against ethanol-induced lesions in mice.[35]

Some NHTP-containing *C*-glycosidic ellagitannins have also been shown to inhibit the human DNA topoisomerase II enzymes (top2). Top2 are nuclear enzymes that are essential for the removal of torsional constraints during replication, chromosome condensation, and segregation by introducing transient DNA double-strand breaks.[36] Top2 are the targets of inhibitors such as doxorubicin or etoposide (VP-16), which stabilize the covalent top2–DNA cleavage complexes and generate permanent double-strand breaks that may ultimately lead to cell death. These drugs are now routinely used for the treatment of a wide range of human cancers.[37] Other inhibitors such as catalytic inhibitors can also inhibit top2 by interfering with the binding between the enzyme and the DNA, by stabilizing DNA–enzyme noncovalent complexes, or by inhibiting ATP binding.[38]

Using the in vitro P4 DNA unknotting assay, Kashiwada and co-workers reported that **1**, **2**, and **11a** were 100- to 250-fold more potent than etoposide (VP-16).[5c] These data gave us the impetus to test other wine-related NHTP-bearing ellagitannins available from this study, including the four novel compounds **9**, **12a**, **12b**, and **21**, for a potential top2 inhibitory activity using the standard kDNA decatenation assay (see Supporting Information). The results that are displayed in Figure 6 and Table 1 show that all ellagitanin derivatives tested inhibit top2-mediated decatenation of kDNA at concentrations as low as 1 μM. When compared to VP-16, which is known to exhibit a low activity in these concentration ranges, the *C*-galloyl glycosidic isocoumarin bergenin, which we recently identified as a selective top2 inhibitor (unpublished results), showed a similar activity. Interestingly, all other ellagitanins showed a much higher activity

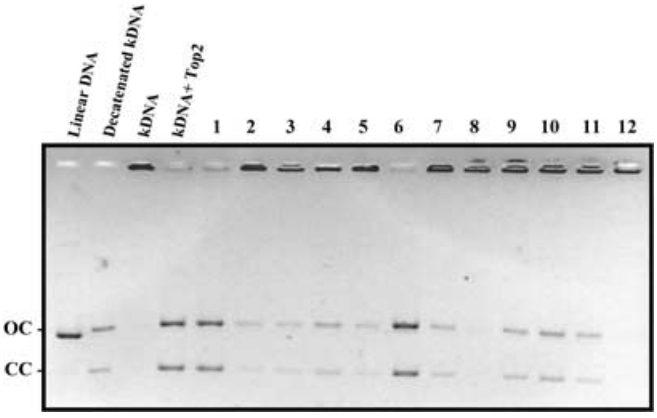



Figure 6. Inhibition of top2-mediated decatenation of kDNA by NHTP-bearing *C*-glycosidic ellagitannins: catenated DNA from kinetoplast (kDNA) was incubated with purified recombinant top2 (170 kDa form) in the absence or in the presence of 1 or 10 μM of the different compounds (see Supporting Information for details). A representative gel is shown for 10 μM concentrations. Lane 1: etoposide (VP-16); lane 2: acutissimin A (**11a**); lane 3: acutissimin B (**11b**); lane 4: epiacutissimin A (**12a**); lane 5: epiacutissimin B (**12b**); lane 6: bergenin; lane 7: β-1-*O*-ethylvescalagin (**9**); lane 8: vescalene (**21**); lane 9: castalagin (**2**); lane 10: vescalagin (**1**); lane 11: castalin (**19**); lane 12: vescalin (**18**). OC and CC correspond to the open circular and the closed circular forms resulting from decatenation of kDNA, respectively. See also Table 1.

Table 1. Inhibition [%] of top2-mediated decatenation for each compound normalized to the amount of decatenated DNA in the top2 lane shown in Figure 6. Values are the mean of at least two independent experiments.

| | VP-16 | **1** | **2** | **9** | **11a** | **11b** | **12a** | **12b** | **18** | **19** | **21** | bergenin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 μM | 3.7 | 55.6 | 62.7 | 49.3 | 89.2 | 78.8 | 72.6 | 74.3 | 95.5 | 87.9 | 78.8 | 4.7 |
| 10 μM | 6.5 | 47.1 | 73.4 | 66.2 | 96.3 | 94.5 | 83.3 | 97.5 | 100 | 67 | 97.3 | 1.3 |

than VP-16 with a *complete* inhibition of top2-mediated decatenation for **18** and **21** at 10 μM concentration.

These results are in accordance with previous reports regarding **1**, **2**, and **11a**,[5c] but showed a significant variation for castalin (**19**), which is active in our experimental setting with approximately 70% inhibition of top2-mediated decatenation for both 1 and 10 μM concentrations. In contrast to VP-16, which is known to induce DNA-top2 covalent cleavage complexes in treated cells, no such complexes could be detected with compounds **1**, **2**, and **11a**.[5c] In fact, **11a** was even shown to partially inhibit the formation of enzyme–DNA complexes in VP-16-treated KB cells, suggesting that these ellagitanins could act as catalytic inhibitors.[38] Because of their structural similarity with **1**, **2**, and **11a**, it is likely that our series of ellagitanin derivatives would also act as top2 catalytic inhibitors. Further studies are in progress to elucidate their precise mechanism of top2 inhibition.

## Conclusion

This work allowed us to unveil for the first time an important aspect of the chemistry of oak-derived NHTP-bearing *C*-glycosidic ellagitannins. These natural products are extracted by the wine solution during aging in barrels and have the capability to combine covalently by means of substitution reactions with a variety of grape-derived nucleophilic species, such as, inter alia, ethanol, flavanols, anthocyanins, and thiols. The particularity of this process is that only *C*-glycosidic ellagitannins bearing a β-oriented hydoxyl group at their C-1 position, exemplified in this study by (−)-vescalagin (**1**), engage in this chemistry with retention of the configuration at C-1. The condensation products thus obtained can evidently contribute to the modulation of wine organoleptic properties. Furthermore, some of these wine ellagic compounds express pharmacologically relevant activities. Based on previously published data,[5c] we were able to confirm that this series of analogous NHTP-bearing ellagitannins, including four novel compounds (i.e., **9**, **12a**, **12b**, and **21**) could target the human topoisomerase II enzyme. Most of the wine-related ellagitannin derivatives we tested were much more potent than etoposide (VP-16) at inhibiting top2-mediated decatenation in vitro, suggesting a potential antiproliferative activity and their potential use as new anticancer drugs. Two previously untested compounds, the known (−)-vescalin (**18**) and the novel vescalene (**21**), fully inhibited top2 at 10 μM concentrations. Moreover, these ellagitannin derivatives have the pharmacological advantage of being highly soluble in water. We are currently investigating whether these species can be used as catalytic inhibitors in specific types of cancer cell lines, or whether they could be used in combination with drugs for the treatment of various malignancies.

## Experimental Section

Detailed descriptions of experimental procedures, HPLC chromatograms, and visible absorbance, electrospray mass, and NMR spectra of all new compounds are given in the Supporting Information.

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