Natural Product Synthesis

DNA Topoisomerase Inhibitor Acutissimin A and Other Flavano-Ellagitannins in Red Wine**

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Polyphenols are ubiquitous in fruits, vegetables, and various plant-derived foods and beverages that have been claimed to be beneficial for human health.[1–3] Among the most evocative examples are red wine, green tea, and chocolate, which all contain polyphenols that are believed to reduce the risk of certain degenerative diseases.[1] The trademark of polyphenols is their antioxidant activity, which puts them center-stage in debates on the prevention of coronary heart disease and atherosclerosis by polyphenol-rich diets. Another widely recognized property of polyphenols is their ability to precipitate proteins through formation of noncovalent complexes. Such complexation of polyphenols with salivary proteins underlies the perception of astringency, a major taste property of red wine and several other beverages.[4] Their potential for application in the food and beverage industry is fueling research ultimately aimed at designing sensors for taste measurement,[4] but the significance of investigations is often limited by lack of access to structurally well-defined polyphenols.

Polyphenols comprise two major classes of natural products: the condensed tannins (proanthocyanidins), which are derived from C–C-linked flavan-3-ols,[1,5,6] and the hydrolyzable tannins (gallo- and ellagitannins), which are derived from galloyl units usually linked by esterification to a sugar core such as glucose.[1,7] Hundreds of purified ellagitannins have been characterized and biologically evaluated as active constituents of plant extracts used in traditional medicine.[7,8] Yet the potential of ellagitannin-based drugs has so far remained untapped, even though pyrogallol-type biaryl and teraryl units (Scheme 1) confer onto this tannin class rigid and stereochemically defined motifs which are well-suited for specific interactions with proteins.[7,9,10]

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Supporting information (detailed descriptions of experimental procedures, liquid chromatograms, and NMR and electrospray mass spectra of compounds **4a**, **4b**, **5a**, **5b**, and mixtures thereof) for this article is available on the WWW under http://www.angewandte.org or from the author.

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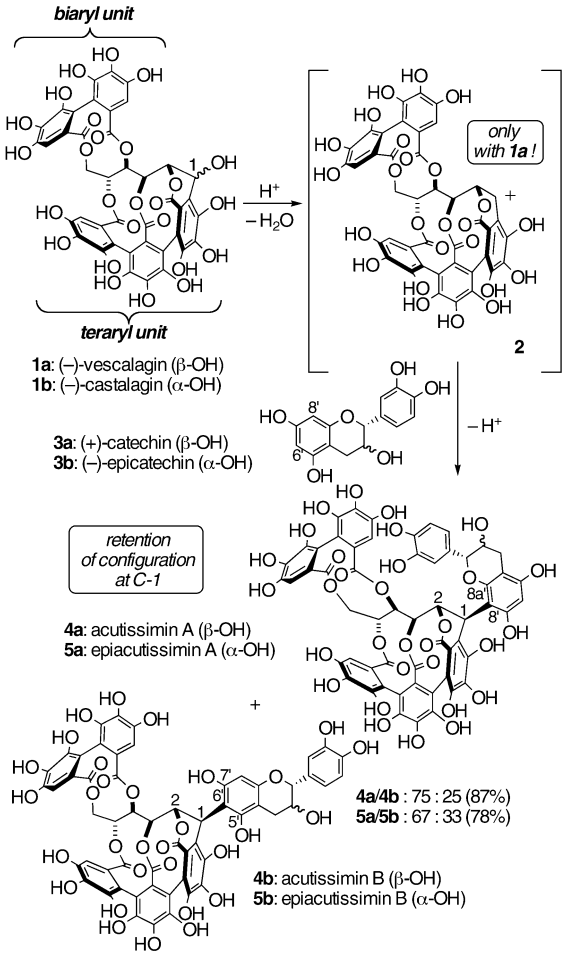

Scheme 1. Formation of acutissimins. Only the *C*-glycosidic ellagitannin (−)-vescalagin (**1a**) reacts under mildly acidic conditions with the flavan-3-ols (+)-catechin (**3a**) and (−)-epicatechin (**3b**) to produce the acutissimins **4a/b** and **5a/b** through a diastereoselective nucleophilic substitution that occurs with retention of configuration at the C-1 center of **1a**.

The work presented herein concerns the formation in red wine of acutissimin A (**4a**) and related flavano-ellagitannins from flavan-3-ols and *C*-glycosidic ellagitannins such as (−)-vescalagin (**1a**) and its C-1 epimer (−)-castalagin (**1b**) that feature an unusual open-chain glucose core C–C-linked to a galloyl-derived teraryl unit (Scheme 1).[11] These *C*-aryl glycosides are characteristic metabolites of durable hardwood species. Acutissimin A (**4a**) was first isolated from the bark of the sawtooth oak (*Quercus acutissima*),[12] a pest- and disease-free oak used as an ornamental tree in air-polluted urban areas in the United States. The compound was later found to be an inhibitor of human DNA topoisomerase II that is 250-fold more potent in vitro (concentration required for 100% inhibition, $IC_{100} = 0.2\ \mu M$) than the clinically used anticancer drug etoposide (VP-16).[13]

The chemistry of the formation of **4a** is simple and involves an acid-catalyzed nucleophilic substitution reaction between either (−)-**1a** or (−)-**1b** at the C-1 center and (+)-catechin (**3a**) at its nucleophilic C-8 center (Scheme 1). Initial attempts by others[12] to use this hemisynthetic approach to produce **4a** from (−)-castalagin (**1b**) resulted in minute amounts (3.7%) of the desired product. The reactions we carried out between purified (−)-**1b** and (+)-**3a** in tetrahydrofuran containing 1% trifluoroacetic acid at 60°C confirmed this failure. However, the use of (−)-**1a** instead of (−)-**1b** led, over a period of 7 h, to the clean formation of acutissimin A (**4a**) as the major product, together with its C-6 regioisomer acutissimin B (**4b**; Scheme 1). Both compounds have the same configuration at C-1 as **1a**. This mixture was then separated by semipreparative HPLC and the individual isomers **4a** and **4b** were obtained in a 75:25 ratio and 87% yield. Interestingly, the isomers have been isolated in a similar ratio (81:19) from *Quercus acutissima*.[12] The identity of the hemisynthetic compounds was confirmed by comparison of ^1H and ^{13}C NMR data as well as optical rotations with published data.[12]

Initial failures to synthesize the target compound were essentially the result of incorrect selection of the starting *C*-glycoside epimer. (−)-Vescalagin (**1a**) is a much more efficient reaction partner than its α-anomer **1b**. This difference in chemical reactivity can be rationalized in terms of the difference in orientation of the reacting hydroxy group at C-1. In **1b**, this OH group is α-oriented and embedded in the *endo* face of the molecule, whereas in **1a**, it is β-oriented and points outward from the *exo* face of the molecule. The latter OH group is consequently more accessible and its oxygen atom probably has a more basic character than that of the OH group in **1b**, which is also involved in intramolecular hydrogen bonding.[14,15]

The same reaction was performed with the flavan-3-ol (−)-epicatechin (**3b**) and gave two new flavano-ellagitannins that we refer to as "epiacutissimins" A (**5a**) and B (**5b**) in a 67:33 ratio and 78% yield, again with retention of configuration at C-1 (Scheme 1). The position at which the flavanoid unit is connected to the *C*-glucoside C-1 center in these two regioisomers was confirmed by the observation of two- and three-bond couplings of H-1 with C-8′ and C-8′a in the NMR HMBC spectrum of **5a**, and with C-5′ and C-6′ in that of **5b**. The stereochemistry at C-1 was deduced from the small NMR coupling constant between the glucose unit H-1 and H-2 protons; this weak coupling indicates that the dihedral angle between these two protons is close to 90° and such an angle is observed when H-1 is β-oriented.[12] In fact, all known naturally occurring C-1-substituted *C*-glycosidic ellagitannins have their C-1 substituent in this β orientation. It is likely that all these compounds are derived from (−)-**1a** and not from (−)-**1b**. A mechanistic description of the reaction follows a classical S_N1-type pathway with protonation of the OH group at C-1 assisting the formation of a benzylic cation **2** (Scheme 1).[1] This stable carbocation intermediate is attacked by the flavan-3-ol units mainly from their C-8 center and, to a lesser extent, from their more encumbered and less nucleophilic C-6 center, with full diastereofacial differentiation (Figure 1).

The hemisynthesis described herein constitutes an in vitro mimicry of the nonenzymatic yet stereoselective formation of acutissimin flavano-ellagitannins. With this sound knowledge of the reactivity of *C*-glycosidic ellagitannins with flavan-3-ols in acidic medium, we turned our attention to the chemistry of red wine because this beverage contains both precursors of

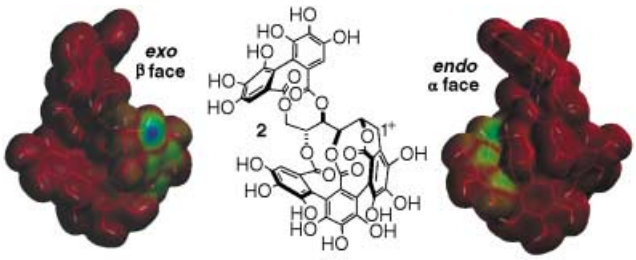

Figure 1. Diastereofacial differentiation of the benzylic carbocation intermediate. These Spartan-generated Hartree–Fock models show the two faces of the lower-energy unoccupied molecular orbital (LUMO) of the benzylic cation **2** mapped onto its 0.002-electron au^{-3} electron density isosurface. The diagrams clearly explain the observed stereochemical preference of the reaction by showing that the carbocation LUMO is indeed more accessible from the exo face (blue spot) than the endo face of the C-1 locus.

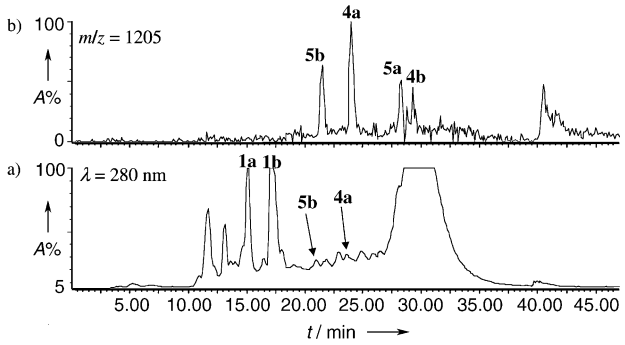

Figure 2. Detection of acutissimins in red wine. HPLC/MS profiles of a partially purified red wine sample. a) UV detection at 280 nm, b) negative-mode electrospray ion trace chromatogram ($m/z = 1205$). **1a**: vescalagin, **1b**: castalagin, **4a**: acutissimin A, **4b**: acutissimin B, **5a**: epiacutissimin A, **5b**: epiacutissimin B.

flavano-ellagitannins. Grape juice brings both catechin (**3a**) and epicatechin (**3b**) to red wine, while vescalagin (**1a**) is extracted from oak by the aqueous alcoholic wine solution during aging in barrels and/or is added by treatment of the wine with enological tannins. We verified that the oak heartwood used to make barrels, from which we isolated **1a** and **1b**,[16] does not contain any detectable amounts of flavano-ellagitannins. Several hundred compounds present in red wine have been characterized so far, so the chances of finding the acutissimins in such a chemically complex medium would have been small without having the compounds already to hand. We first carried out a model reaction by mixing **1a** and **3a** at room temperature in a wine model consisting of a 12% ethanolic aqueous solution containing 5 $g L^{-1}$ tartaric acid at pH 3.2. Formation of both acutissimins **4a/b** was clearly shown by HPLC and electrospray mass spectrometric analysis over a period of 25 days; once again, **4a** was the major product. These results confirmed our hypothesis that acutissimins can be found in red wine and the only remaining challenge was to develop an appropriate extraction protocol (see the Supporting Information). The two acutissimins **4a/b** and the two previously unknown "epiacutissimins" **5a/b** were detected in a sample of red wine that had been aged for 18 months in an oak barrel. Each of the four compounds has a molecular mass of 1206 Da, and their presence was validated by mass spectrometry and comparison of their chromatographic retention times and mass fragmentation patterns with those of the hemisynthetic compounds (Figure 2).

While it would be quite inappropriate to infer from the presence of acutissimin A in red wine that this beverage possesses antitumor properties, our work shows for the first time that wine contains polyphenolic molecules displaying both ellagitannin and flavanoid structural features. These hybrid tannin molecules are accessible through the hemisynthesis reported herein and should be part of molecular-level studies on the effects of polyphenol–protein interactions. Furthermore, the efficacy of the chemistry involved led us to speculate that higher-molecular-mass flavano-ellagitannins similarly constructed from oligomeric *C*-glycosidic ellagitannins and proanthrocyanidins are present in red wine. The amounts of acutissimins in an aging red wine at any given time can appear rather low when measured by any available method, but it should be remembered that wine is a slow but continuously evolving mildly acidic and aerobic reaction mixture. The acutissimins are certainly transformed in wine during aging, but they will form as long as grape-derived flavan-3-ols and oak-derived vescalagin are present in the medium.

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[1] E. Haslam, *Practical Polyphenolics—From Structure to Molecular Recognition and Physiological Action*, Cambridge University Press, Cambridge, **1998**.
[2] T. Hatano, T. Yoshida, R. W. Hemingway in *Plant Polyphenols 2: Chemistry, Biology, Pharmacology, Ecology* (Eds.: G. G. Gross, R. W. Hemingway, T. Yoshida), Kluwer Academic/Plenum, New York, **1999**, p. 509.
[3] M.-T. Huang, C.-T. Ho, C. Y. Lee, *Phenolic Compounds in Food and their effects on Health*, Vol. II, *ACS Symp. Ser. 507*, American Chemical Society, Wahington, DC, **1992**.
[4] A. Edelmann, B. Lendl, *J. Am. Chem. Soc.* **2002**, *124*, 14741.
[5] D. Ferreira, D. Slade, *Nat. Prod. Rep.* **2002**, *19*, 517.
[6] A. P. Kozikowski, W. Tückmantel, G. Böttcher, L. J. Romanczyk, *J. Org. Chem.* **2003**, *68*, 1641.
[7] S. Quideau, K. S. Feldman, *Chem. Rev.* **1996**, *96*, 475.
[8] T. Okuda, *Curr. Org. Chem.* **1999**, *3*, 609.
[9] D. R. Spring, S. Krishnan, H. E. Blackwell, S. L. Schreiber, *J. Am. Chem. Soc.* **2002**, *124*, 1354.
[10] K. Saeki, S. Hayakawa, M. Isemura, T. Miyase, *Phytochemistry* **2000**, *53*, 391.
[11] G.-I. Nonaka, T. Sakai, T. Tanaka, K. Mihashi, I. Nishioka, *Chem. Pharm. Bull.* **1990**, *38*, 2151.
[12] K. Ishimaru, G.-I. Nonaka, I. Nishioka, *Chem. Pharm. Bull.* **1987**, *35*, 602.
[13] Y. Kashiwada, G.-I. Nonaka, I. Nishioka, K. J.-H. Lee, I. Bori, Y. Fukushima, K. F. Bastow, K.-H. Lee, *J. Pharm. Sci.* **1993**, *82*, 487.
[14] T. Yoshida, H. Ohbayashi, K. Ishihara, W. Ohwashi, K. Haba, Y. Okano, T. Shingu, T. Okuda, *Chem. Pharm. Bull.* **1991**, *39*, 2233.
[15] N. Vivas, M. Laguerre, Y. Glories, G. Bourgeois, C. Vitry, *Phytochemistry* **1995**, *39*, 1193.
[16] A. Scalbert, L. Duval, S. Peng, B. Monties, C. Du Penhoat, *J. Chromatogr.* **1990**, *502*, 107.

