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Review

Recent progress in ellagitannin chemistry

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Abstract

Continuing studies on the total synthesis of ellagitannin plant metabolites have led to the preparation of the dimeric antitumor compound, coriariin A, as well as designed structural analogues. In related investigations, the synthesis of a 2,4-hexahydroxydiphenoyl (HHDP)-bearing glucopyranose structure has been achieved. This species is related to the geraniin family of ellagitannins, and its subsequent chemistry is suggestive of a mechanistic rationale for the observation that the HHDP units within (3,6-bridged)2,4-HHDP-containing ellagitannins invariably are oxidized further in vivo. Companion studies designed to assay the immunomodulatory properties of coriariin A and analogues have led to the thesis that tumor necrosis factor alpha (TNFα) serves as a mediator of this ellagitannin's tumor remissive activity. Furthermore, certain tannins and tannin analogues appear to act in an immunosuppressive capacity with peripheral blood monocytes that were exposed to the bacterially derived septic shock inducing agent lipid A.

Keywords: Ellagitannins; Chemical synthesis; Coriariin A; Geraniin; Immunomodulation; Atrop-selective

Contents

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1. Introduction

The chemistry and biology of ellagitannins remains an active concern at the Pennsylvania State University, and this review article will update progress and identify future thrusts in this area of research since the last review of our group's work, published in conjunction with the 3rd Tannin Conference held in Bend Oregon, July 1998 (Feldman et al., 1999a). Our goals in the tannin area remain unchanged: (1) to develop a toolbox of chemical techniques for the construction of key structural features within the ellagitannin framework, (2) to apply these techniques to the synthesis of representative ellagitannins and designed analogues, and (3) to employ these materials as probes for the elucidation of the biological mechanism-of-action that underlies the sometimes profound immunomodulatory properties of certain members of this class of plant metabolites. Within these broad goals, decisions about program direction have had to be made, and the intermittent successes and more frequent failures over the years have helped refine our perspective and focus our efforts on the following specific projects:

(a) the total synthesis of coriariin A (**1**, Fig. 1);
(b) the synthesis of coriariin A analogues;
(c) the synthesis of 2,4-hexahydroxydophenoyl (HHDP)-containing glucopyranose structures in order to explore the question of why there are no 3,6-bridged 2,4-HHDP bearing naturally occurring ellagitannins, despite the identification of numerous 3,6-bridged species with an oxidized HHDP unit spanning positions C(2) and C(4) (e.g., geraniin (**2**), Fig. 1);
(d) identification of the intrinsic immune system agent that mediates the tumor remissive properties of coriariin A;
(e) exploration of the thesis that tannin constructs might down-regulate as well as up-regulate certain immune responses and hence provide useful leads for the design of anti-sepsis agents.

The studies described below will address these issues, and in so doing, present a clearer picture of the promise, and the problems, that this class of molecules holds for both chemistry and biology.

2. Brief summary of previous work from the author's laboratory at the Pennsylvania State University

Prior efforts from our group were directed toward the development of both C–C and C–O bond forming strategies that permitted joining of two galloyl units in a regiochemically and stereochemically predictable fashion, Scheme 1, **3** → **4** and **3** → **5**, respectively (Feldman and Ensel, 1994a; Feldman et al., 1996a). At the outset of our work, a survey of the literature revealed that successful formation of these entities was limited to low-yielding coupling of simple galloyl esters (or gallic acids) using enzymic oxidation for HHDP formation (Mayer et al., 1984) or Ullmann-type chemistry in the case of digalloyl ethers (Mayer, 1952; Lin et al., 1990). Neither of these technologies held much promise for application to the more complex and sensitive substrates anticipated by our natural product synthesis goals, and so we recognized early on that new directions were required.

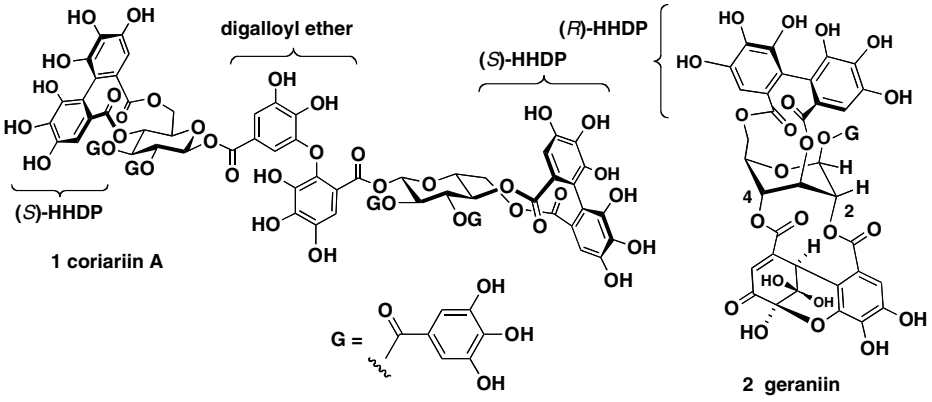
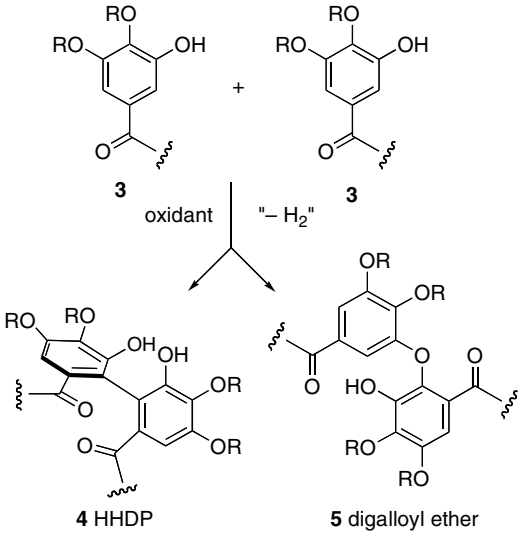


Fig. 1. Representative ellagitannins; examples of HHDP and digalloyl ether units.

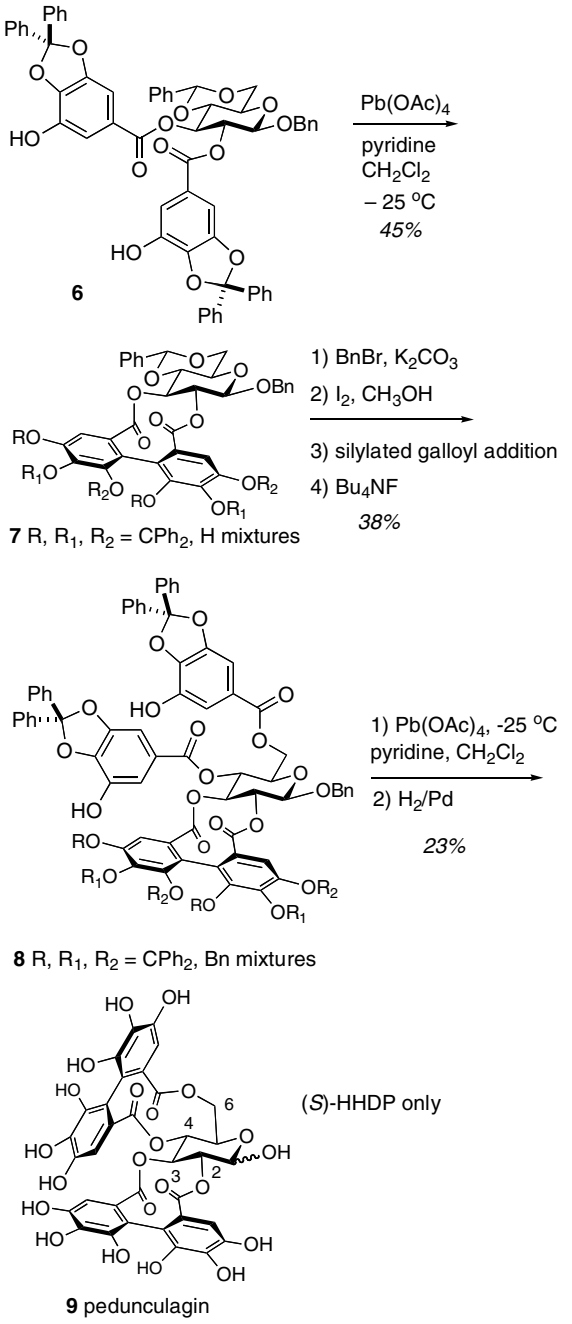


Scheme 1. Galloyl oxidative coupling.

2.1. HHDP synthesis

Earlier reports by Schmidt and then Haslam, which laid the foundation for rationalizing the observed diastereoselectivity emerging from a postulated oxidative coupling of glucose-bound galloyl esters upon ellagitannin biosynthesis, proved exceedingly influential (Schmidt and Mayer, 1956; Gupta et al., 1982). These hypotheses suggested that if we could only identify an oxidant that would mediate the galloyl coupling, stereochemistry would take care of itself. The appeal of this biomimetic approach to atrop-selective HHDP formation could not be resisted, and so we screened innumerable phenolic oxidants in the hopes of discovering a reagent that would couple two glucose-bound galloyl units without subsequent destruction of the HHDP product by overoxidation, a major confounding process observed in earlier ellagitannin work. This prospecting venture eventually yielded a suite of metal-based oxidants that performed as desired, to greater or lesser extents, and the best procedure utilized a variant of the venerable Wessely oxidation [$Pb(OAc)_4$, base, low temperature] (Bubb and Sternhell, 1970) to deliver the intact glucose-bound HHDP moiety. Our efforts are exemplified by the total synthesis of pedunculagin (**9**) from the galloylated glucose precursor **6**, Scheme 2 (Feldman and Smith, 1996b).

The basis for avoiding product overoxidation in the Wessely procedure remained mysterious at the outset. Subsequent mechanistic work provided evidence supporting a scenario wherein the pivotal C-C bond forming event occurs in an Sn2′-like manner upon nucleophilic attack by one galloyl group onto an adjacent galloyl now rendered quite electrophilic by formation of an intermediate Ar–$OPb(OAc)_3$ function (Feldman and Hunter, 1998). Product oxidation would



Scheme 2. The synthesis of pedunculagin (**9**).

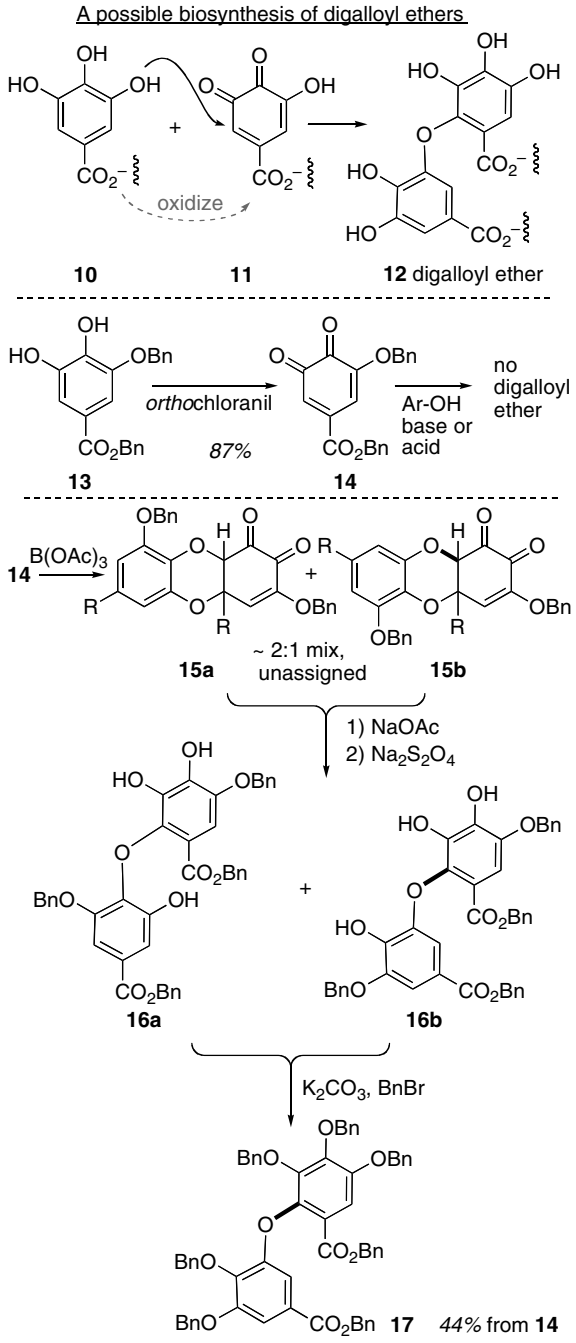
be dissuaded in this "anchimeric assistance" mechanistic proposal by virtue of the fact that it would be exceedingly difficult to juxtapose a third nucleophilic galloyl alongside a putative lead-activated HHDP, an arrangement apparently necessary to consummate the C–C bond forming process. For example, phenol-containing HHDP products could be recovered unchanged upon brief exposure to Wessely oxidation conditions. The stereochemical outcomes of these C(4)/C(6) and C(2)/C(3) oxidative coupling reactions are in complete accord with the predictions of the Schmidt–Haslam hypothesis, later refined by molecular mechanics (MM)-based

(Mohamadi et al., 1990) analysis of substrate conformations (Quideau and Feldman, 1996). This calculational approach to rationalizing/predicting glucose-bound galloyl coupling stereoselectivity has proven invaluable in guiding substrate design within this project, and recent X-ray studies (vide infra) validate its application in this context.

The lead-based oxidative cyclization protocol is quite robust and general for HHDP synthesis. The monomeric ellagitannins tellimagrandin I (Feldman et al., 1994b), tellimagrandin II (Feldman and Sahasrabudhe, 1999b), sanguiin H-5 (Feldman and Sambandam, 1995) and pedunculagin all have been prepared via this chemistry, and the lessons learned from these successful exercises provide the foundation for our approach to the more challenging dimeric target coriariin A, as described below. While our studies were ongoing, a conceptually different approach to stereoselective digalloyl coupling on glucopyranose substrates was described by Martin (Dai and Martin, 1998). This stereoselective Ullmann-based reductive coupling chemistry of iodogalloyl esters complements our efforts in that oxidative-sensitive substrates can be accommodated, but at present the synthesis of only the permethyl ethers of the HHDP units has been reported. A completely different perspective on ellagitannin synthesis was adopted by Khanbabaee (Khanbabaee and van Ree, 2001; Khanbabaee and Grober, 2003), who has prepared several monomeric members of this class of plant metabolites by glucose diol esterification with a preformed, protected HHDP unit. This latter strategy was predicated upon seminal contributions by Meyers (Nelson and Meyers, 1994), Lipshutz (Lipshutz et al., 1994), and Itoh (Itoh et al., 1996), and can be gainfully employed with either a chiral version of the HHDP module, or through kinetic resolution of the racemic HHDP variant.

2.2. Digalloyl ether synthesis

The chemistry developed to fashion the key C–O bond (emboldened) in digalloyl ether **17** followed a less direct, but still arguably biomimetic, strategy, Scheme 3 (Feldman et al., 1996a). Initial efforts to pattern a synthesis approach on the putative biosynthetic sequence **10** + **11** → **12** failed completely. Galloyl *ortho*-quinones of the type **14** were readily accessible and tractable, but all attempts to induce these soft electrophiles to participate in nucleophilic capture by a phenolic addend led instead to low yields of a C–C bonded HHDP product. This mismatch in nucleophile (hard): electrophile (soft) pairing eventually was overcome by resorting to an indirect series of reactions initiating with a non-regioselective dimerization of *ortho*-quinone **14**, mediated by a weak Lewis acid. This nominal Diels–Alder transform establishes the critical C–O bond (emboldened in **15b**), but it also



Scheme 3. Digalloyl ether synthesis based upon galloyl *ortho*-quinone chemistry.

introduces a second unwanted C–O connection, and the lack of regioselectivity was discouraging. However, both of these deficiencies could be overcome by simply treating the mixture of Diels–Alder adducts with base (β-elimination of the extraneous C–O bond), reductant (nascent *ortho*-quinone to catechol), and then base/benzyl bromide. This final step proved key in correcting the poor regioselectivity of the Diels–Alder reaction, because the mild base was sufficient to promote

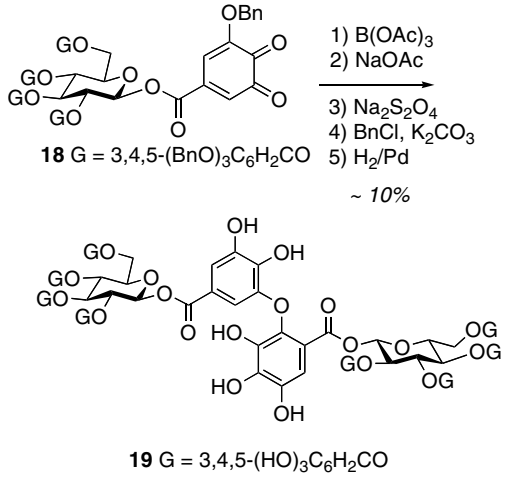
a Smiles rearrangement (Yoshida et al., 1992, 1993) of the undesired isomer **16a** into the requisite regioisomer **16b**. Presumably, this formal 1,2-shift is driven by the convergence of both steric (**16b** has an *ortho*-hydrogen; **16a** does not) and electronic factors (the phenoxide anion derived from **16b** is stabilized by the *p*-ester; in **16a** the anion cannot be so stabilized).

This sequence of reactions was suitable for delivering the digalloyl ether of simple esters (e.g., methyl, benzyl). Unfortunately, it did not export to the more complex glucopyranose *ortho*-quinone system **18**, and only modest amounts of the gallotannin/ellagitannin hybrid **19** could be obtained even after extensive optimization studies, Scheme 4 (Feldman and Sahasrabudhe, 1999b; Feldman et al., 2000). A possible rationale for the disparate behaviors of **14** and **18** might cite the fact that **14**, by chance, precipitates out of solution as it is formed, leading to pure material as input into the Diels–Alder reaction. The *ortho*-quinone **18** does not enjoy this fortuitous purification, and its Diels–Alder sequence does not benefit from starting with pure material. It appears that the digalloyl ether forming chemistry is not as general as the C–C bond forming HHDP chemistry. This limitation proved costly in the initial approach to coriariin A.

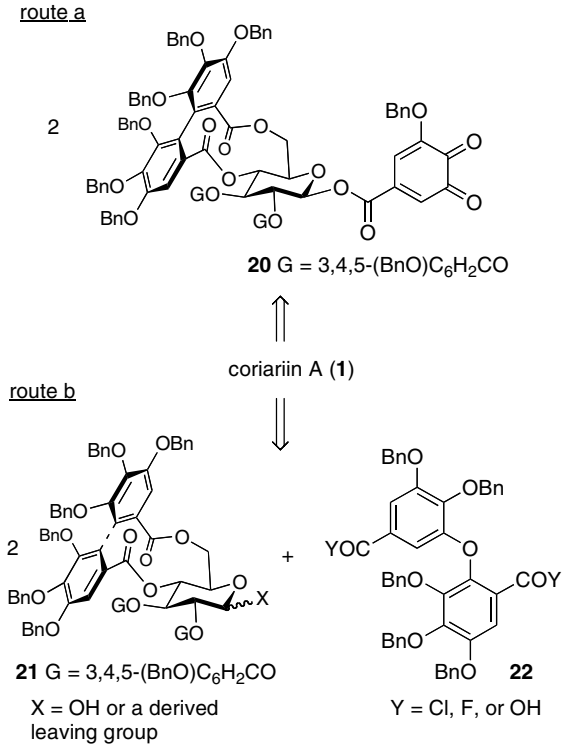
3. Coriariin A (1) synthesis

The coriariin A (**1**) synthesis began with the premise that the HHDP-forming and digalloyl ether-forming methodology will be applicable to this more complex target. The modular nature of coriariin A′s structure suggested that the sequence of C–C and C–O galloyl coupling steps did not have to be set at the design stage, but rather could be responsive to emerging results. Thus, two distinct a priori strategies were contemplated, Scheme 5. Route (a) features a C–C first approach, and presumably hews closest to the presumptive biosynthesis of coriariin A via tellimagrandin II oxidative dimerization. Route (b) embodies the C–O first approach, as the digalloyl ether moiety is introduced intact. The heightened convergency of route (a) and its biomimetic appeal were sufficient to elevate it to the top priority, but with the sure knowledge that the capriciousness of the galloyl *ortho*-quinone dimerization chemistry must be confronted late in the route. The more secure chemistry of route (b) then serves as a fallback position in the event that route (a) becomes problematic.

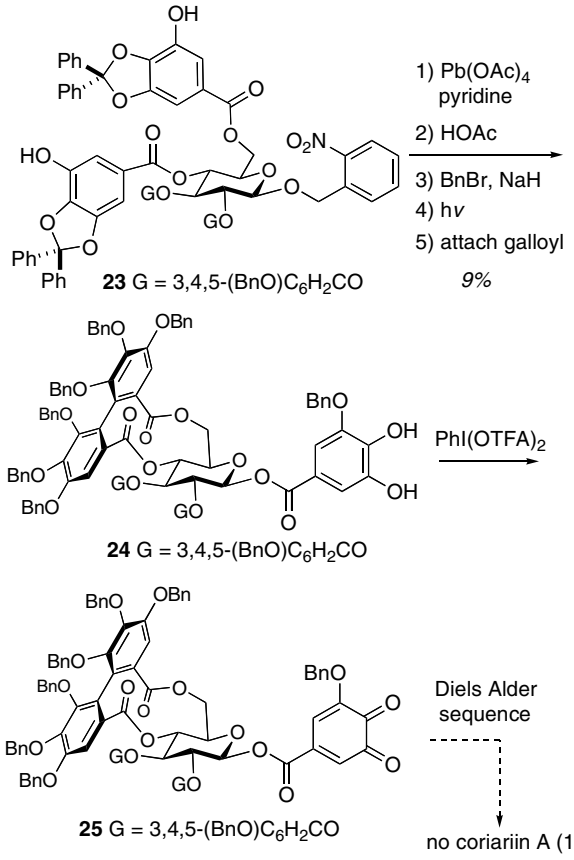
The key tellimagrandin II derivative **25** was assembled by application of the Wessely oxidative cyclization chemistry, Scheme 6 (Feldman et al., 2000). Oxidation of the catechol function within **24** led to an isolable *ortho*-quinone **25** that could not, however, be purified by chromatography or crystallization. The purest sample of **25** was obtained by performing the oxidation with $PhI(OTFA)_2$ at −40 °C, which furnished the *ortho*-quinone contaminated only by residual PhI after workup and removal of volatiles. Other oxidants (*ortho*-chloranil, $PhI(OAc)_2$, Ag_2O, CAN) did provide the *ortho*-quinone as well, but with more uncharacterized impurities. Subjection of the tellimagrandin II "monomer" **25** to the Diels–Alder dimerization sequence described earlier, followed by global hydrogenolytic debenzylation, led to a complex mixture of products from which no coriariin



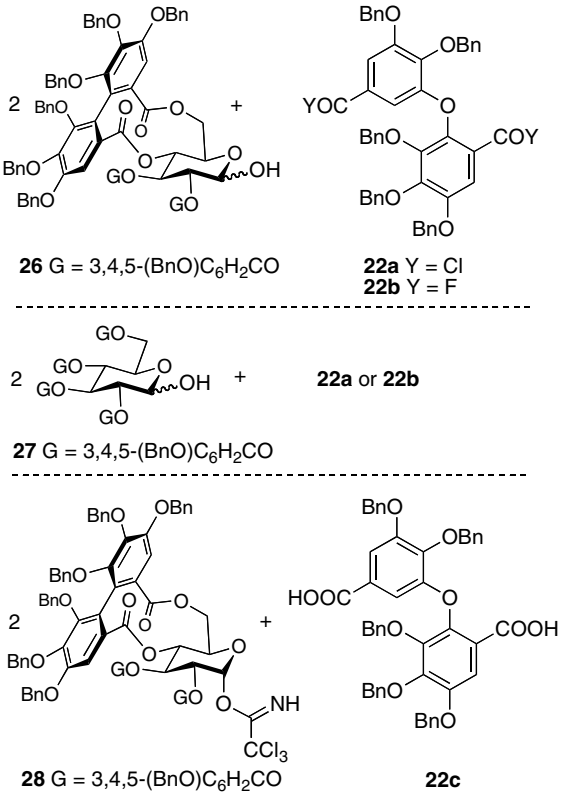
Scheme 4. Attempt to apply the Diels–Alder dimerization chemistry to a glucopyranose-based system.



Scheme 5. Two retrosynthetic strategies for coriariin A (**1**).



Scheme 6. A first attempt at coriariin A (**1**) via possibly biomimetic tellimagrandin II dimerization.

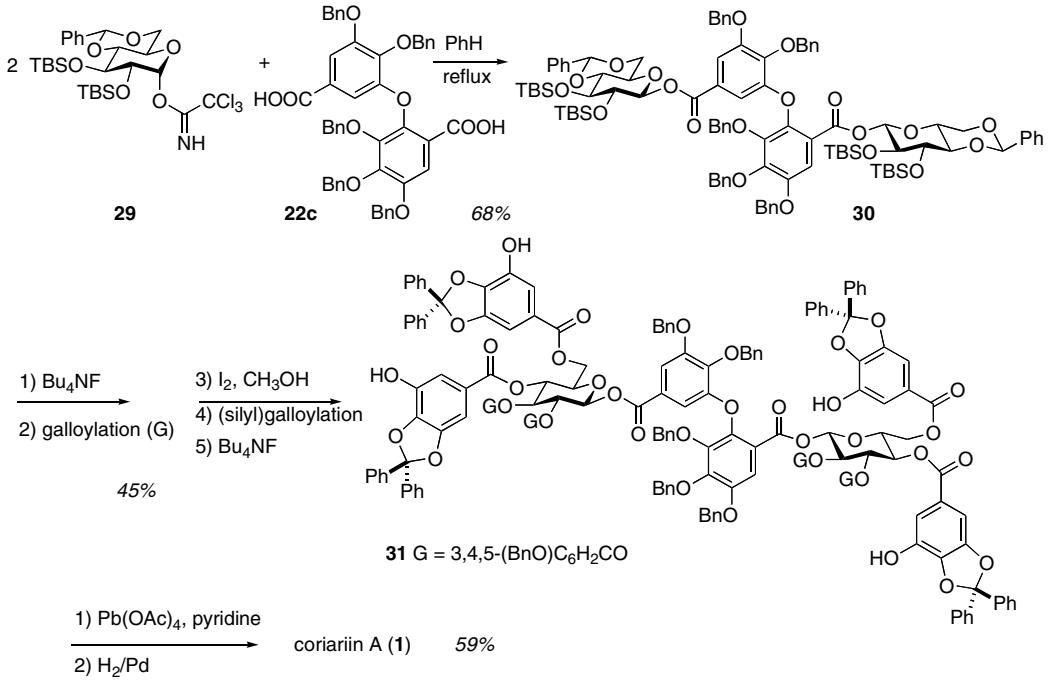


Scheme 7. Failed attempts at three-component condensations to form the coriariin A framework.

A (**1**) could be identified. An authentic sample in hand provided a reliable comparison point for NMR and chromatographic assays, and it is fair to conclude that if any coriariin A (**1**) was present, it could not have been more than 1% or 2% of the crude reaction mixture. Thus, the inverse relationship between substrate complexity and product yield for the *ortho*-quinone Diels–Alder chemistry, hinted at earlier, is reinforced by these experiments. At this juncture, the biomimetic tellimagrandin II dimerization strategy [Scheme 5, route (a)] was abandoned in favor of the longer but presumably more reliable route (b).

The three-component fragment coupling approach to coriariin A (**1**) [route (b)] required the means to connect the preformed digalloyl ether unit to two glucopyranose cores with stereoselectivity for the β anomers. At the outset, this transformation was not expected to be challenging in light of the numerous successful glucopyranose galloylations accomplished during the formative stages of this project. That this naïve assumption was spectacularly wrong should have come as no surprise by this point. Unexpected acylation failures (Scheme 7) turned out to be the norm with the digalloyl ether moiety when compared to similar reactions with simple gallic acid derivatives. Direct acylation attempts in the tellimagrandin I series **26** with either the *bis* acid chloride **22a** or its fluoride analogue **22b** did not furnish any anomeric ester products. Similarly, the simple uncoupled galloylated glucopyranose derivative **27** failed to capture either **22a** or **22b**. A switch in acylation mechanism to a nucleophilic carboxylate/electrophilic glucopyranose did not provide any initial reason for optimism. The trichloroacetimidate **28**, which combined readily with simple tri-O-benzyl gallic acid, did not participate in a Schmidt-type acylation (Schmidt and Jung, 1997) with the digalloyl ether *bis* acid **22c**. However, seminal observations by Fraser-Reid regarding the relationship between glucopyranose C(1) electrophilicity and the electronic character of the C(2) and C(3) substituents provided a new perspective on the problem (Fraser-Reid and Madsen, 1997). Perhaps the inductively electron-withdrawing C(2) and C(3) galloyl esters on substrate **28** place too much electron demand on C(1), thereby muting its reactivity with the apparently only weakly nucleophilic carboxylates derived from **22c**. If this hypothesis has merit, then a course of action presents itself: replace the electron withdrawing C(2) and C(3) esters with electron releasing groups and increase the electrophilicity of C(1).

This plan was reduced to practice with the *bis* silyl ether glucopyranose substrate **29**, available from glucose itself in 5 steps, Scheme 8. It was gratifying to find that



Scheme 8. Completion of the coriariin A (**1**) synthesis.

this species did indeed react in a Schmidt-type acylation with diacid **22c** to afford the three-component product **30** in good yield. Only the *bis* β (equatorial) isomer was detected, an observation in accord with the expected mechanistic course of the Schmidt procedure. Digalloyl ether **30** was still quite distant from a useful coriariin A (**1**) precursor, and so a series of routine protecting group manipulations were required to arrive at tetraphenol **31**, a substrate for *bis* Wessley reaction. The generality of this HHDP-forming transform was reinforced by the smooth conversion of this functionally rich substrate **31** into a *bis* HHDP-containing precursor to coriariin A. Upon exposure to hydrogenolytic debenzylation conditions, this complex mixture of regioisomers converged cleanly and in satisfactory yield to the natural product coriariin A (**1**).

This route was suitable for preparation of over 100 mg of coriariin A (**1**), in the aggregate, from many runs. This material, along with the gallotannin/ellagitannin hybrid **19** prepared by similar chemistry, fueled the subsequent biological studies described below. In addition, this synthesis effort is once again a reminder of the all too common dictum in natural products synthesis that simple model compounds do not simply model complex systems. The reason(s) for the lack of reactivity of the digalloyl ether diacid and derivatives when compared to monomeric gallic acid derivatives remains obscure.

The (*S*)-HHDP stereochemical outcome of the Wessely oxidative cyclization of **31** was expected based upon much precedent and the aforementioned Schmidt–Haslam hypothesis. This postulate, however, does not identify the structural features of the galloylated glucopyranose system that provide the means to transduce starting material conformational information into product stereochemistry. MM calculations can bridge this gap, as exemplified by the in silico reaction sequences **32a** → **33a** and **32b** → **33b**, Fig. 2. Thus, β-pentagalloylglucose (**32**) can exist in two low-energy conformations that differ by the tilt of the galloyl rings with respect to the glucopyranose plane. The rotational isomer **32a** is calculated to be about 1 kcal/mol lower in energy than the alternative tilt isomer **32b**. To the extent that this ground-state energy difference is expressed in the transition state for C–C bond formation, the (*S*)-HHDP-containing product **33a** is expected, provided that bond formation occurs between the set of diastereotopic carbons that are closest together within **32a**. This model was self-consistent, but lacked independent confirmation.

An X-ray crystallographic analysis of *per*-*O*-methyl-β-pentagalloylglucopyranose was pursued in order to provide some measure of validation for this calculational model (Powell, 1999). The results of this study are shown as structure **34** in Fig. 2. The crystal packing diagram indicates that a molecule of toluene is intercalated between the galloyl rings at C(1) and C(2), whereas the galloyl rings at C(2) and C(3) are splayed apart as a consequence of an interdigitated C(2) galloyl unit from an adjacent molecule of **34** in the crystal. Only the C(4) and C(6) galloyl rings appear to reside in locations unperturbed by crystal packing events. The C(4) and C(6) galloyl groups are juxtaposed in almost perfect

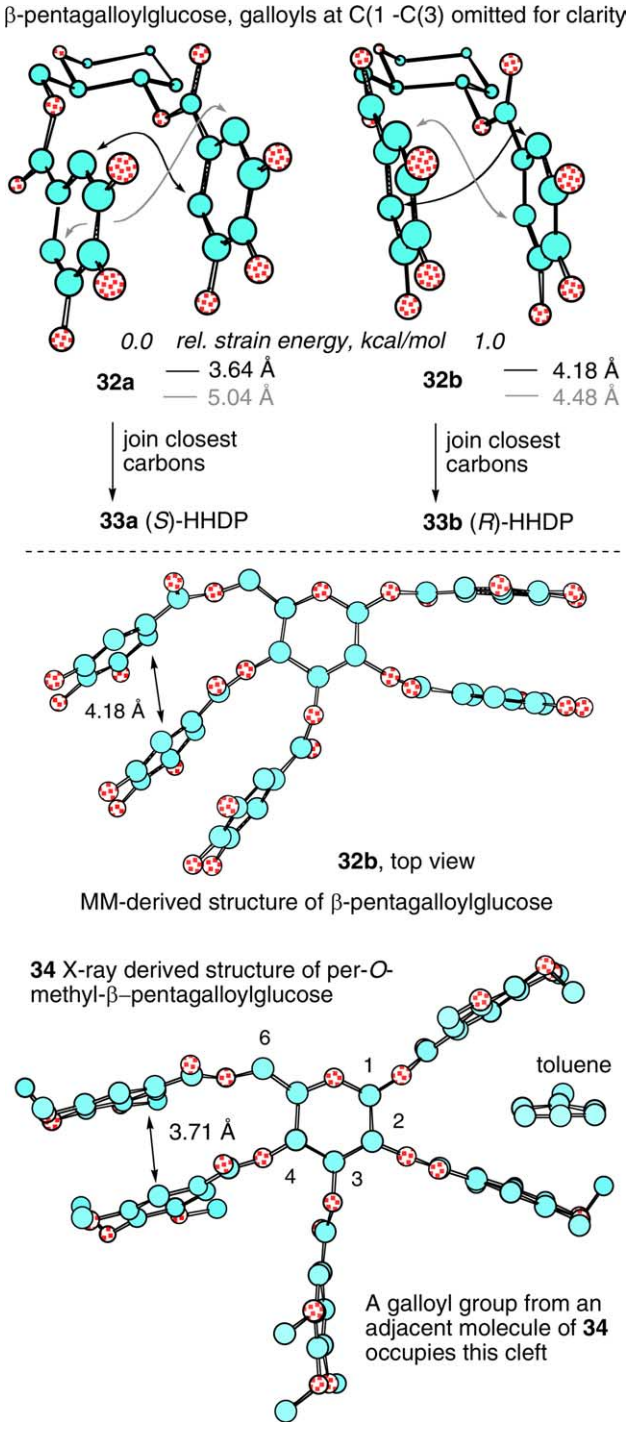


Fig. 2. Molecular mechanics and X-ray analysis of β-pentagalloylglucose structure.

parallel alignment, with an inter-ring distance (3.71 Å) consistent with close-packing of the π clouds. The aryl rings are, however, offset as a result of a counterclockwise tilt relative to the glucopyranose core. Consequently, the two sets of diastereotopic inter-ring carbons that might, in principle, participate in oxidative cyclization span distinctly different distances (3.71 Å vs. 5.09 Å). It is pertinent to note that the two carbonyl groups of the C(4) and C(6) galloyl esters are aligned in parallel.

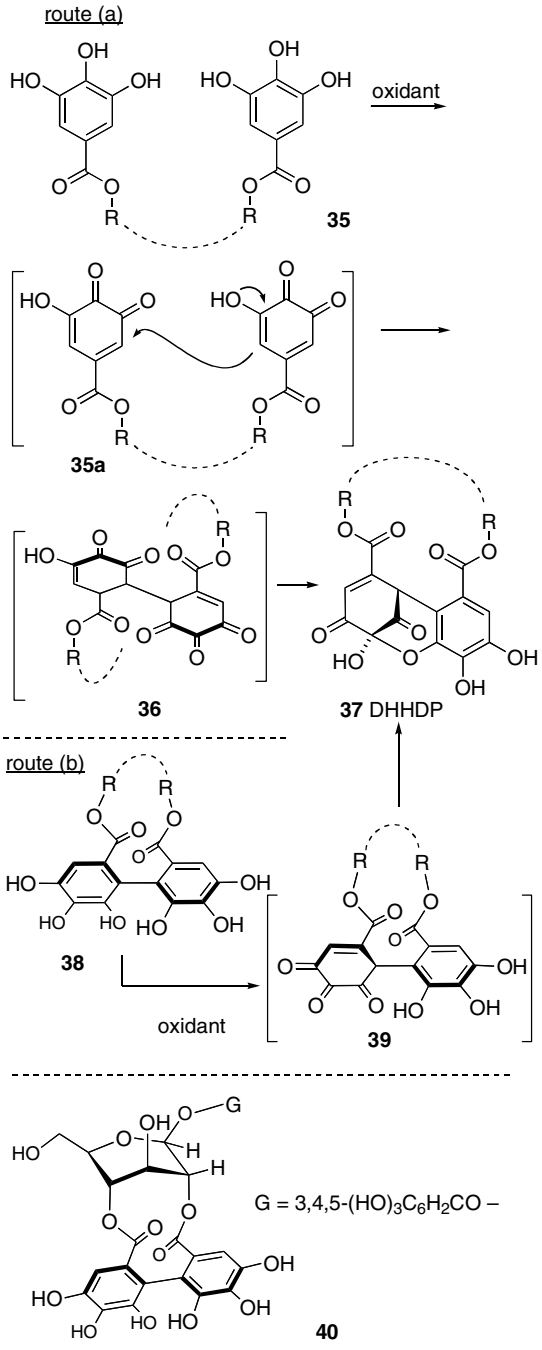
Evidently, *per-O*-methyl-β-pentagalloylglucose does not crystallize in the MM-suggested lowest energy conformation (≈**32a**), plausibly as a result of compensatory crystal packing forces. Nevertheless, the similarities between the experimentally measured parameters in **34** and the analogous ones in the computationally derived species **32b** (cf. the distances indicated in Fig. 2) support use of an MM-based model for analyzing cyclization stereoselectivity.

4. 2,4-HHDP-containing glucopyranose synthesis

Our synthesis effort directed toward the geraniin family of ellagitannins offered two new challenges in HHDP construction: (1) formation of an (*R*)-HHDP atropisomer spanning the C(3)/C(6) glucopyranose junction, and (2) formation of an oxidized (i.e., dehydrohexahydroxydiphenoyl, DHHDP) version of the biaryl unit bridging carbons 2 and 4 of the glucopyranose core. The all-axial disposition of the substituents on geraniin (**2**) raises the degree of difficulty considerably for any strategy that proceeds through simple galloylated glucose precursors, as these appendages must be held in a thermodynamically unfavorable position prior to C–C bond formation. The studies described below focus on the second of these two tasks (2,4-bridging biaryl).

In principle, two independent oxidative cyclization protocols for DHHDP construction can be envisioned, Scheme 9. In route (a), oxidation of the fully phenolic galloyl units in **35** might generate the transient *ortho*-quinones of **35a**, reactive species that could participate in conjugate addition as shown to deliver the DHHDP unit in **37** after tautomerization and hemiketal closure. Alternatively, route (b) follows a more conservative path in which the HHDP unit initially is installed through reliable Wessely oxidation chemistry, and then in a second oxidation step, the HHDP moiety is further modified to furnish an *ortho*-quinone intermediate **39** capable of cyclizing to the DHHDP product **37**. In both sequences, product (over)oxidation may be an issue. Both plans have been explored, but the isolation of a glucose-bound DHHDP-containing species still remains elusive.

A description of the route (a) chemistry is covered in an earlier review (Feldman et al., 1999a). The results of those studies can be summed up by noting that the chemistry proceeds as shown with **35** → **35a** for simple methyl gallate (R–R = CH_3, CH_3), but attempts to extend this successful DHHDP synthesis to a glucopyranose-bound digallate system (**35**, R–R = 4,6-glucopyranose) were not rewarded. In those cases, complex product mixtures with all of the signatures of

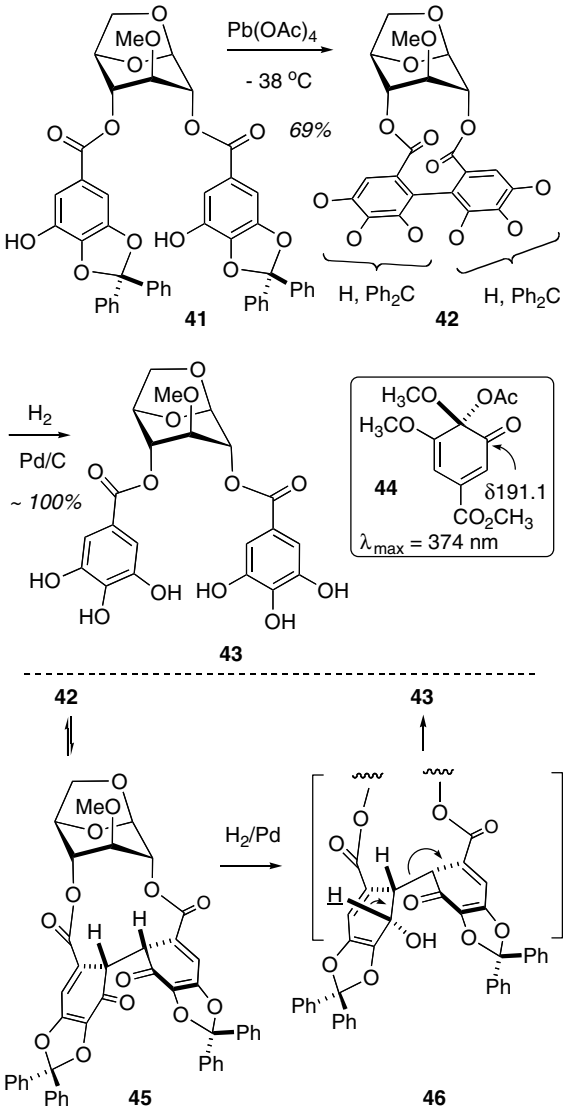


Scheme 9. Synthesis strategies for DHHDP synthesis.

polymerization invariably were observed. Apparently, solubility is the key, as the sparingly soluble methyl ester DHHDP product **37** (R–R = CH_3, CH_3) precipitates out of solution before it can be destroyed by further reaction, whereas the more complex glucopyranose substrate does not enjoy this advantage and then is consumed by further reaction (oxidation?). Route (b) offers a potential solution in this regard. Since the C–C bond forming and *ortho*-quinone generating steps are now distinct, it may be possible to identify an oxidant that is reactive enough to process **38** into **39**, but not so reactive that the product **37** will be destroyed.

The formation of a C(2)/C(4)-bridging HHDP system raises some interesting questions in light of the proposed biosynthesis of geraniin (**2**) (Tanaka et al., 1986). The geraniin family of ellagitannins contains over 15 structurally related members that all have the C(3)/C(6) (*R*)-HHDP unit and an oxidized/modified HHDP unit spanning C(2) and C(4). No C(3)/C(6) bridged ellagitannin natural products that feature a simple, unoxidized HHDP group at C(2)/C(4) have been identified, despite the hypothesis that such a species is a candidate for a biosynthesis precursor. Curiously, there is a single C(2)/C(4)-HHDP-containing ellagitannin, but this natural product, phyllanemblinin B (**40**), does not have the C(3)/C(6) bridge. These observations beg the related questions, "What is so privileged about phyllanemblinin B, and why hasn't a C(2)/C(4)-HHDP-containing precursor to geraniin (**2**) been identified?".

The exploration of these issues, all within the context of geraniin model system synthesis, began with the galloylated anhydroglucose derivative **41**, Scheme 10 (Feldman et al., 2003). This substrate bears a C(1)/C(6) linkage as an imperfect model for geraniin's C(3)/C(6) bridge, but on the functional level, it does enforce an axial disposition of the galloyl groups at C(2) and C(4). Wessely oxidation proceeds in good yield to afford the presumed HHDP-containing species **42** as a complex mixture of regioisomers. Application of standard hydrogenolysis conditions was expected to deliver the fully phenolic HHDP product in an uneventful transformation. That this expectation was not met became readily apparent when the reaction product was shown to be the Ar–Ar cleavage species **43**. Hydrogenolytic cleavage of an Ar–Ar bond, if indeed occurring, would be an entirely unprecedented reaction. This unusual result forced a reexamination of the crude Wessely oxidation product, starting with chromatographic purification to remove what were presumably trace amounts of an orange impurity. At this juncture, a second surprise became evident: the orange "impurity" was not an impurity at all, but rather an integral part of the product mixture, which still tallied about six discrete but structurally related compounds. This mixture's UV/VIS absorbance (λ_{max} = 386 nm) and ^{13}C NMR spectrum (weak ketone peaks at δ 191.5 and 190.7) suggested the presence of at least some of a cyclohexadienone-containing species (cf. **44**), shown for simplicity as the single tautomer **45**. In light of this proposal, the formal Ar–Ar cleavage observed upon hydrogenolysis of "**42**" can be rationalized by citing reaction through cyclohexadienone **45** to give a reduced intermediate **46** that can suffer β-elimination of the appended aryl ring ($-H^+$) and regenerate the two uncoupled aryl cores of **43**. The implication of this postulated mechanism is that cyclohexadienone **45** is in



Scheme 10. Synthesis of a nominal C(2)/C(4)-HHDP-containing geraniin model.

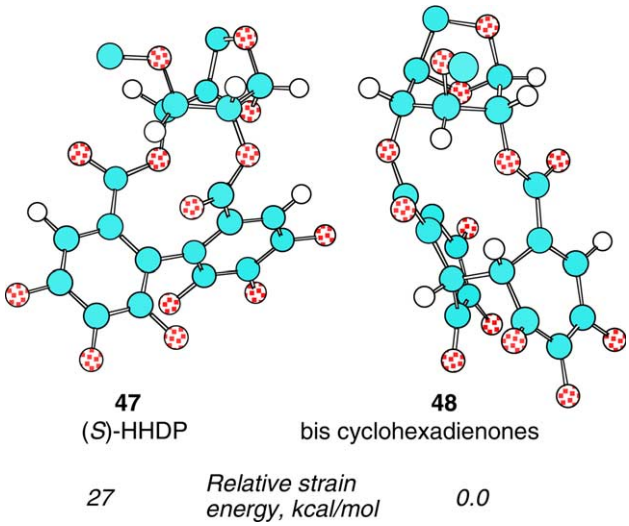


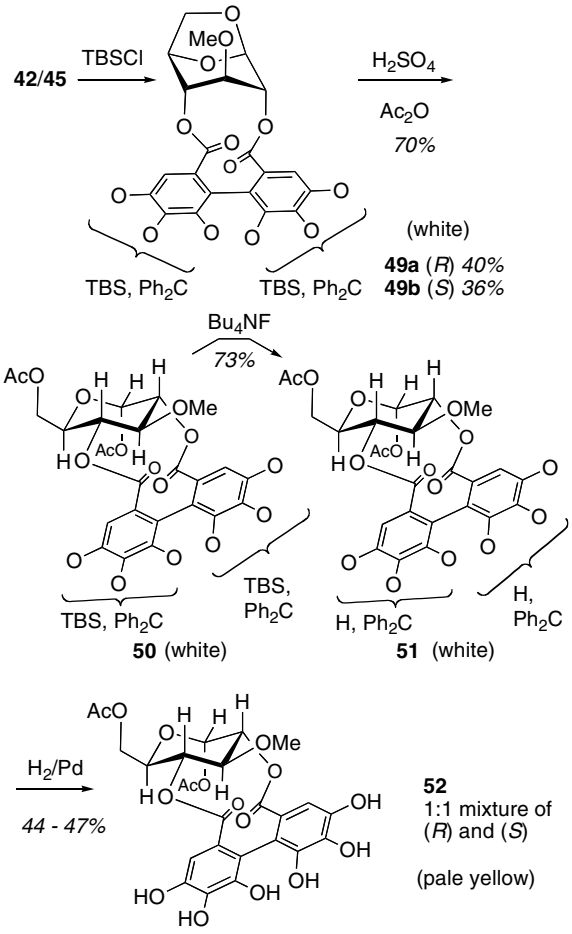
Fig. 3. Molecular mechanics analysis of the C(2)/C(4) HHDP tautomers.

facile equilibrium with **42**, and so all of the mixture **42** funnels through **45** en route to **43**.

Why should normally stable aromatic rings prefer to exist, at least in part, as non-aromatic cyclohexadienone tautomers? Some insight into this question can be gleaned from the results of MM calculations, Fig. 3. The results of this computer modeling suggested that the highly strained C(1)/C(6)-bridged C(2)/C(4)-HHDP-containing system **47** is substantially higher in energy than its cyclohexadienone tautomers, exemplified by the lowest energy species **48**. The weight of these observations, taken together, begins to speak to the question of geraniin (**2**) biosynthesis posited earlier. Perhaps any C(3)/C(6)-bridged, C(2)/C(4)-HHDP-containing species is so inherently unstable that it has no more than a fleeting existence, and is readily oxidized (or otherwise modified) through its highly reactive cyclohexadienone tautomeric form. In contrast, the reactivity of **45** is likely diminished somewhat by the diphenyl ketal protecting groups, thus permitting isolation.

The odd case of the uniquely stable C(2)/C(4)-HHDP-containing ellagitannin phyllanemblinin B raises questions about the role of the C(3)/C(6) bridge in influencing the stability of the geraniin system's C(2)/C(4)-biaryl moiety. To answer these questions, we sought the means to cleave this bridge without destroying the HHDP unit. After much trial and error, a procedure that involved (1) silylation of the free phenols, (2) brief acid treatment, and (3) in situ acylation, delivered the C(1)/C(6) bisacylated product **50** as a mixture of at least six compounds, Scheme 11. The initial silylation of the orange mixture **42/45** led to a *white bis* silyl ether mixture, an observation that gains significance in light of the proposed **42/45** equilibration. Two major pure silyl ether isomers **49a** and **49b** were isolated from this 4-component mixture in 40% and 36% overall yields, respectively. Circular dichroism spectra of these pure compounds displayed the patterns typically assigned to (*R*)- and (*S*)-HHDP units, respectively, indicating that the Wessely oxidative cyclization sequence delivered a non-atropselective product. Control experiments demonstrated that these two atropisomers did not interconvert under the experimental conditions. It is not possible at present to assign a mechanistic rationale to this lack of selectivity, as hypotheses based upon (1) (kinetic) non-selective Wessely oxidative cyclization, or (2) (thermodynamic) product equilibration via **45**, cannot be distinguished.

In any event, the acquisition of the ring-opened glucopyranose mixture **50** opened up the possibility of preparing, for the first time, a fully phenolic C(2)/C(4) HHDP-containing ellagitannin construct. Desilylation of this white mixture of isomers led to an equally



Scheme 11. Completion of a phyllanemblinin B model system synthesis.

colorless mixture of six isomeric *bis* phenolic HHDP-containing species **51**. It is significant within the context of the proposed **42/45** equilibration to note that this mixture of bis phenols was white, and no evidence (UV, ^{13}C NMR) for a cyclohexadienone tautomer was forthcoming. It appears that cleavage of the constraining C(1)/C(6) bridge of **42** removes the driving force for HHDP tautomerization, a conclusion consistent with the observed stability of the non-bridged ellagitannin phyllanemblinin B. The final test of this hypothesis came when removal of the remaining diphenyl ketal protecting groups by hydrogenolysis was attempted. In this instance, the transformation proceeded without event, and the six isomers of **51** converged to give an equilibrating mixture of two fully phenolic HHDP-containing species, the (*R*)- and (*S*)-HHDP atropisomers **52**. These pale yellow compounds could be isolated as distinct species following hydrogenolysis of pure (*R*)- and (*S*)-HHDP containing diphenyl ketal precursors, but they equilibrated to a ~1:1 mixture after 24 h at room temperature. The mechanism of this equilibration (Ar–Ar bond rotation or tautomerization to an unobserved cyclohexadienone intermediate) remains uncertain.

And so, it is not unreasonable to suggest that the chemistry of **42** and **52**, and by analogy geraniin (**2**) and phyllanemblinin B, is dominated by the strain placed on the C(2)/C(4) HHDP group by the rigid C(1)/(C6) bridge. The overarching goal of geraniin DHHDP synthesis via a species such as **52** can now be addressed. Preliminary results are not encouraging. Treatment of **52** with oxidant has not yet furnished any isolable/characterizable material. It would be premature to dismiss this approach based upon these scouting experiments, but a solution to the likely problem of product overoxidation may be required to make further progress.

5. Biological assays

5.1. Immunostimulation by tannin constructs

The entry point for our biological studies of ellagitannin chemistry can be traced to a series of papers by Miyamoto and Okuda that documented the promising tumor remissive properties of several complex ellagitannin structures, including coriariin A (**1**) (Miyamoto et al., 1987a,b, 1993; Murayama et al., 1992). This seminal work described how some dimeric ellagitannins promoted tumor necrosis in various mouse xenograft models at concentrations well below toxic levels. Furthermore, biological mechanism-of-action studies by Miyamoto led to a proposal that the ellagitannins were operating indirectly through the intermediacy of some endogenous effector (Murayama et al., 1992; Miyamoto et al., 1993). A causative role tentatively was assigned to the cytokine interleukin 1β (IL-1β) in this capacity. At the time that these studies were executed, the multifaceted activities of IL-1β were just beginning to be characterized, and speculation that this immune system messenger had (indirect) antitumor capabilities was not unreasonable. However, in the decade or so since these preliminary reports, much effort has been directed towards elucidating the many functions of IL-1β in vivo, and it appears that tumor remission is not, in fact, among its purview (Abbas et al., 1997).

Our research program in this area started with the premise that an immune system component distinct from IL-1β was responsible for the observed tumor remissive properties of the dimeric ellagitannins. A great deal of immunostimulatory/anticancer research since Miyamoto's work has converged on the thesis that the related cytokine tumor necrosis factor alpha (TNFα) is the complicit biological mediator in immune system mediated tumor cell death (Old, 1985). An in-depth mechanistic understanding of this complex process is still being refined. TNFα itself has been explored as an antitumor therapeutic, but systemic toxicity has proven

too difficult to overcome (Spriggs and Yates, 1992; Sidhu and Bollon, 1993; Corti and Marcucci, 1998). In a few studies, localized administration of TNFα has been an effective antitumor therapy, but this limited application requires species circumstances for success (Sidhu and Bollon, 1993; Hieber and Heim, 1994; Lejeune, 1995; Eggermont et al., 1996; Fraker et al., 1996). Initial secretion of TNFα has been implicated in later release of IL-1β, and, if TNFα was indeed the responsible endogenous mediator in the tannin studies, perhaps it was this secondary cytokine discharge that Miyamoto et al. detected in their assays.

We chose to examine TNFα secretion from human peripheral blood mononuclear cells (hPBMC's) following Miyamoto's experimental protocols (Feldman et al., 1999c). ELISA kits were used to quantitate the level of cytokine released, using lipopolysaccharide (LPS, active ingredient = lipid A) as a positive control and an untreated cell sample as a negative control. Preliminary experiments with the gallotannin/ellagitannin hybrid **19** probed two fundamental questions: (1) can a tannin structure elicit TNFα release from hPBMCs?, and (2) what is the time dependence of this release? The data shown in Fig. 4 allow the first question to be answered in the affirmative, and demonstrate that maximal TNFα secretion appears to occur at about 24 h after exposure to **19**. Both positive and negative controls attest to the validity of this method for assaying tannin-induced TNFα release from hPBMCs.

These encouraging results prompted dose–response studies with a wider range of tannin structures, Fig. 5 (Feldman et al., 1999c). The data illustrate that over the low μM concentration range, both coriariin A (**1**) and the hybrid structure **19** are similarly effective at stimulating TNFα release from hPBMC's. The monomeric species β-pentagalloylglucose (**32**) is much less potent in this regard, especially at the lower concentration ranges. Again, positive (LPS) and negative (blank) controls bracketed these data (not shown). This limited structure/activity study revealed two significant pieces of information: (1) dimeric tannin constructs are much more effective than related monomeric species at stimulating TNFα secretion from hPBMCs, and (2) C(4)/C(6) galloyl coupling (HHDP formation) is not essential for biological activity. This latter observation gains significance in light of the much more arduous synthesis required to access the HHDP-containing dimer coriariin A compared to the galloyl uncoupled species **19**.

A second locus of synthesis difficulty in the dimeric tannin series is the digalloyl ether unit. A brief study of its contribution to biological activity was undertaken in the hopes that active structures bearing more readily accessible linker units could be identified, Fig. 6 (Feldman et al., 2002). Replacing the digalloyl ether linker unit with simple aromatic (**53a–c**) or alkyl (**53d**) groups led to materials that were completely ineffective at inducing TNFα release from hPBMCs. In fact, just **53d** amongst these non-digalloyl ether entries provided any response at all, however weak. Only the digalloyl ether-containing structure **53e**, shorn of **19**'s phenoxyl units, displayed any TNFα inducing ability, albeit at a level only about one third of that of the parent compound **19**. These data are consistent with a structural model for the biological activity of pergalloylated diglucopyranose constructs that features very stringent requirements for the digalloyl ether linker unit, but much more relaxed requirements along the molecular periphery.

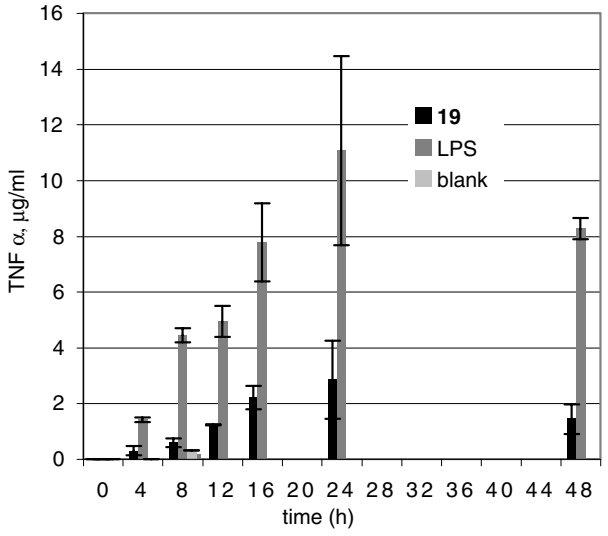


Fig. 4. Time–response data for the secretion of TNFα from hPBMCs treated with the gallotannin/ellagitannin hybrid 19, LPS, and no additive (blank).

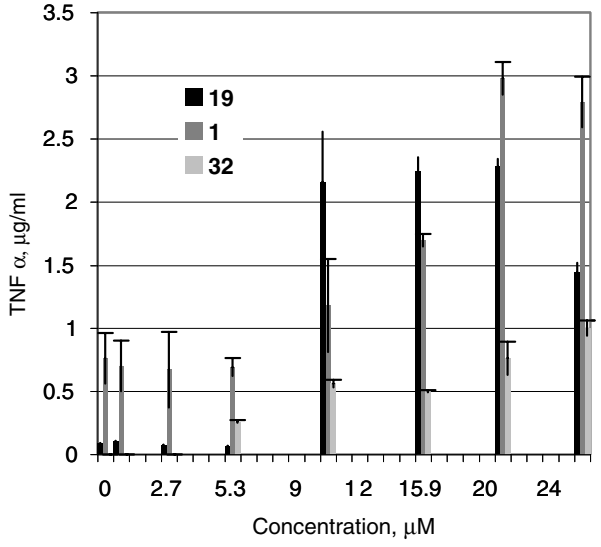


Fig. 5. Dose–response data for the secretion of TNFα from hPBMCs treated with coriariin A (**1**), the gallotannin/ellagitannin hybrid **19**, and β-pentagalloylglucose (**32**).

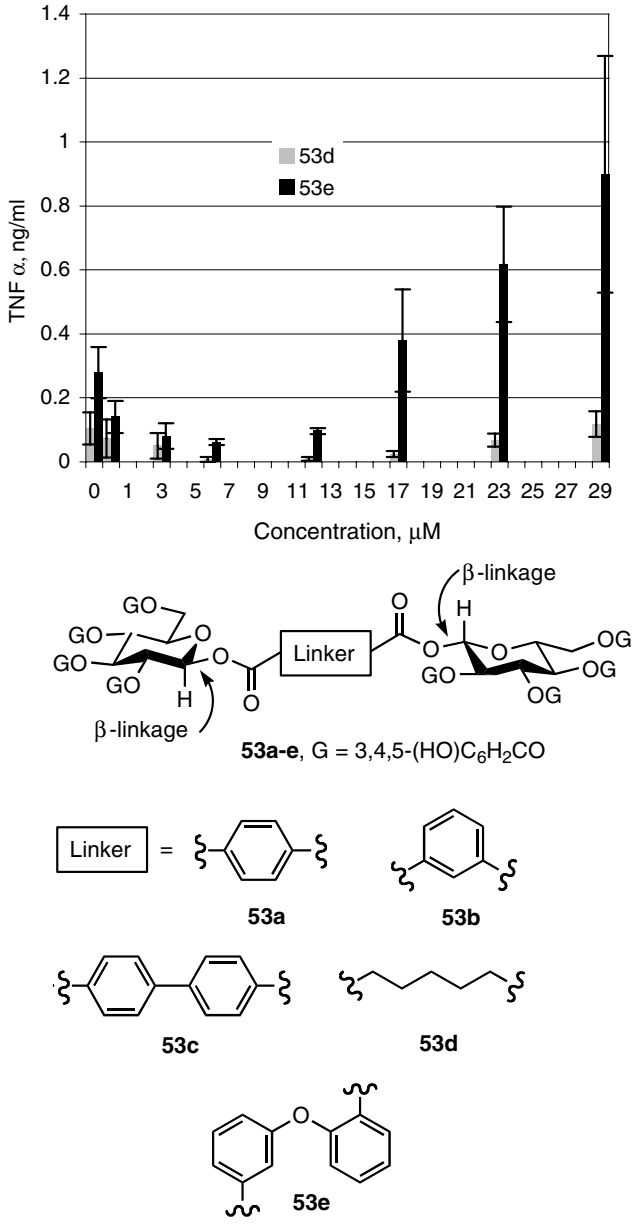


Fig. 6. Dose-response data for the secretion of TNFα from hPBMCs treated with the modified linker tannin analogues **53a–e**.

Speculation about the molecular mechanism by which the dimeric tannins might exert their immunostimulatory activity was guided by consideration of the dose–response profiles of LPS (lipid A) when compared to these species. Consistently similar dose–response data between these two disparate classes of structures (cf. **54**, Fig. 7) led to the thought that perhaps both types of immunostimulators were utilizing the same cellular receptor system. The lipid A receptor system in mammals has been well characterized, and current art in this area is summarized in Fig. 7 (Schumann et al., 1990; Heumann et al., 1992; Dentener et al., 1993; Steinemann et al., 1994; Ulevitch and Tobias, 1995; Misra et al., 1996; Stancovski and Baltimore, 1997; Poltorak et al., 1998). The essential elements of this complex model begin with recognition of lipid A, released from the destruction of gram-negative bacteria, by the serum soluble receptor lipopolysaccharide binding protein (LBP). This protein delivers lipid A to the monocytes' surface-bound receptor CD14, which itself has no nuclear signaling capability. However, in a poorly understood process, this three-part complex, when assembled correctly, engages and activates several other membrane-bound proteins, including toll-like receptor 4 (Tlr-4). Activated Tlr-4 initiates a signal transduction cascade that passes through the inhibitor of NF-κβ en route to TNFα gene transcription. Several downstream events must then occur correctly in order to release TNFα from the monocytes in its biologically active form. Alternative receptors/pathways that bypass CD14 have been identified, especially at high lipid A concentration, and the relative importance of these different TNFα-generating triggers is uncertain at present (Loppnow et al., 1995; Blondin et al., 1997; Malhotra and Bird, 1997).

Exposure to lipid A is implicated in the deadly disease bacterial sepsis (septic shock), whose physiological hallmark is a systemic overproduction of the cytokines IL-1β and TNFα. LPS-induced bacterial sepsis remains the number one cause of death in hospital Intensive Care units, and it is responsible for over 200,000 fatalities a year in the United States (Malhotra and Bird, 1997; Sharma and Dellinger, 2003). The need for anti-sepsis medicines is acute, and the complex nature of the lipid A stimulation cascade has provided several venues for attempted chemotherapeutic intervention. As indicated in boxes in Fig. 7, therapies designed to interrupt lipid A/CD14 interactions, therapies to inhibit the kinase-dependent signaling pathway, therapies to block TNFα precursor cleavage, and therapies to sequester mature TNFα have all been explored, but little progress has been reported to date (Newton and Decicco, 1999; Sharma and Dellinger, 2003).

Tannin constructs may be uniquely poised to make a contribution to this active area of research. The dimeric nature of the active tannins, and their similar dose–response date compared to LPS, raise the possibility that perhaps a dimeric tannin mimics lipid A in its role as the focal point of LBP/CD14 receptor heterodimerization. It is not critical that the tannin duplicates lipid A's (currently unknown) binding motif, but rather all it must do is organize the receptor complex in an orientation capable of recruiting Tlr-4 and associated proteins. This proposed lipid A-tannin connection delves deeply into the realm of speculation, and experimental tests remain for the future. Nevertheless, framing the tannin mechanism-of-action hypothesis in terms of lipid A agonism has the advantage of suggesting a strategy for designing a lipid A antagonist from a tannin structure.

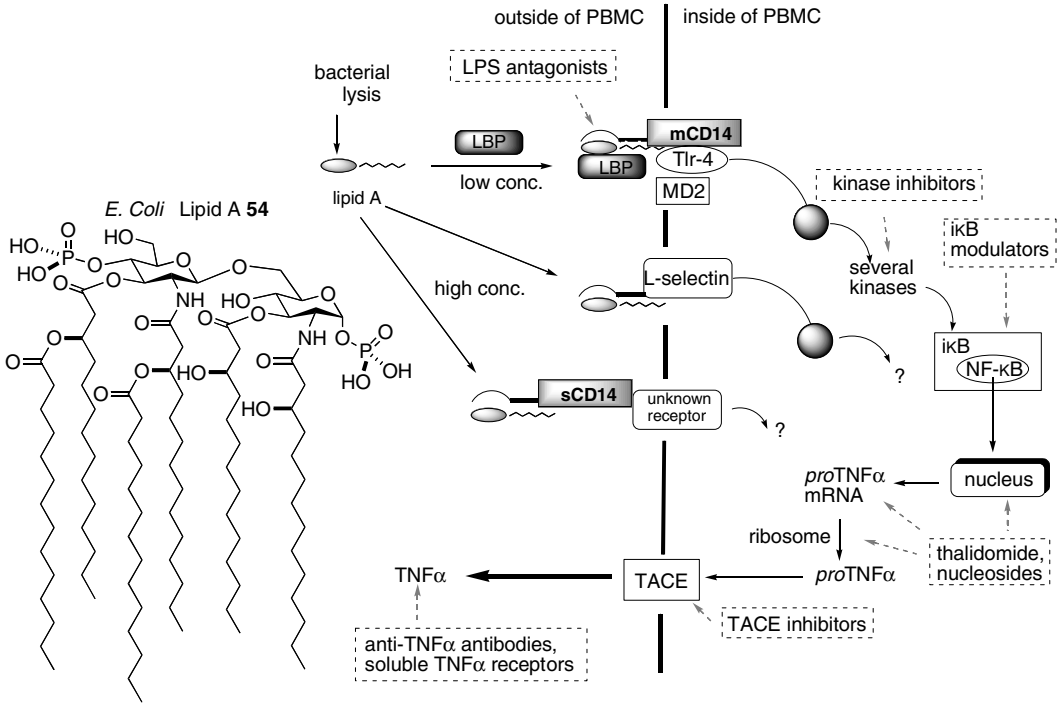


Fig. 7. Schematic of the lipid A-initiated mechanism of TNFα release from PBMCs (adapted from Triantafilou and Triantafilou, 2002).

5.2. Immunosuppression by tannin constructs

If the active dimeric tannins are indeed agonists of lipid A, then it may be possible to modify their structure to (1) preserve receptor binding, but (2) misalign the receptor complex and prevent signal transduction. If inactive tannin binding is competitive with lipid A, then an antagonist will be in hand. The study described with Fig. 6 revealed that the digalloyl ether linker is crucial for biological activity, but we can now pose the question, is it crucial for receptor binding? It is this premise that was tested with the series of dimeric gallotannin analogues **53a**–**d** and the simple monomer β-pentagalloylglucose (**32**) (=a linkerless "dimeric" tannin). Exposure of three different individuals' PBMCs to LPS led to the expected high levels of TNFα secretion. Subsequent treatment of these "septic" cells with increasing amounts of β-pentagalloylglucose (**32**) had the dramatic effect of significantly lowering the TNFα output, Fig. 8 (Feldman et al., 2001). Taken at face value, these data speak, for the first time, to the possibility that tannin structures might indeed play a role in the development of chemotherapeutic anti-sepsis strategies. Of course, observing the expected outcome of a hypothesis by no means validates that hypothesis, and so the mechanistic implications of these data remain to be elucidated. This encouraging result, however, did prompt further exploration of the tannin-as-antagonist thesis with the analogues **53a**–**d**.

β-Pentagalloylglucose itself would not be an effective anti-sepsis agent, since at therapeutic levels it too can stimulate some TNFα and IL-1β release (cf. Fig. 5). On the other hand, the dimer tannin analogues **53a**–**d** did not share this burden, as all were ineffective at eliciting TNFα secretion (cf. Fig. 6). The question then can be posed, are any of these "dead" compounds capable of antagonizing lipid A activity? In vitro studies with **53a**–**d** revealed that substantial inhibition of LPS-mediated TNFα secretion could be achieved, Fig. 9 (Feldman et al., 2002). In all cases, TNFα levels dropped by one third to one half of their original values when 5–10 μM of the tannin construct was added to LPS-stimulated-hPBMC's.

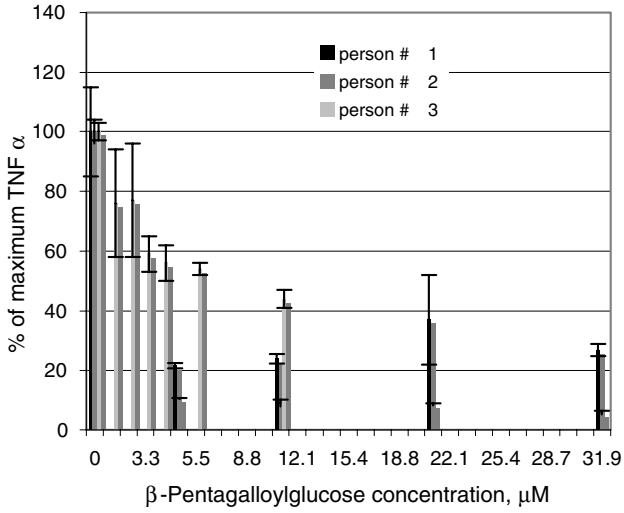


Fig. 8. Dose–response data for the β-pentagalloylglucose-mediated inhibition of LPS-stimulated TNFα release from hPBMCs for three subjects.

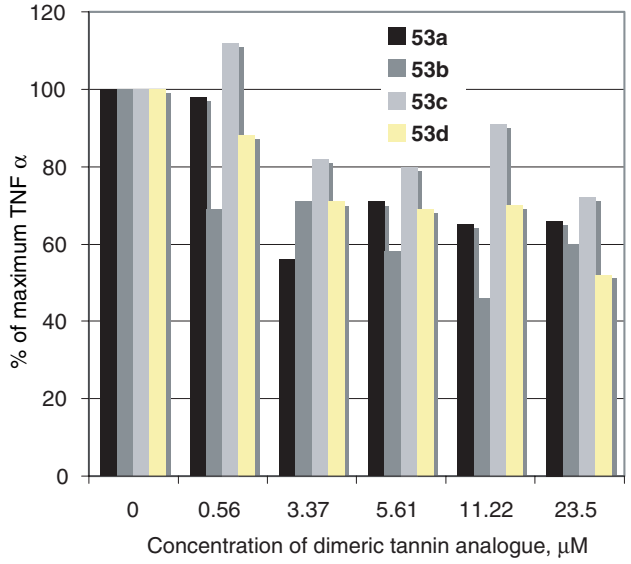


Fig. 9. Dose–response data for dimer tannin analogue-mediated inhibition of LPS-stimulated TNFα release from hPBMCs.

These promising in vitro results motivated pursuit of more relevant in vivo studies using live rats as septic shock models (Feldman et al., 2002). Dimeric tannin analogues **53c** and **53d** were selected as candidates for these in vivo studies based upon their performance in the in vitro trials (Figs. 6 and 9). Nine rats was catheterized and a dose of 1 mg/kg of LPS was administered to eight of them (9th rat = saline control). Two rats each then were treated with either the biphenyl linked dimer **53c** or the alkyl chain linked analogue **53d** (20 mg/rat), and blood plasma TNFα levels were assayed at 90 min after dosing. The data (Fig. 10) show that the biphenyl linked species **53c** reduced circulating TNFα levels by approximately 58% compared with the untreated rats. The alkyl chain linked dimer **53d** performed similarly with one rat, but the second rat showed little decrease compared with untreated

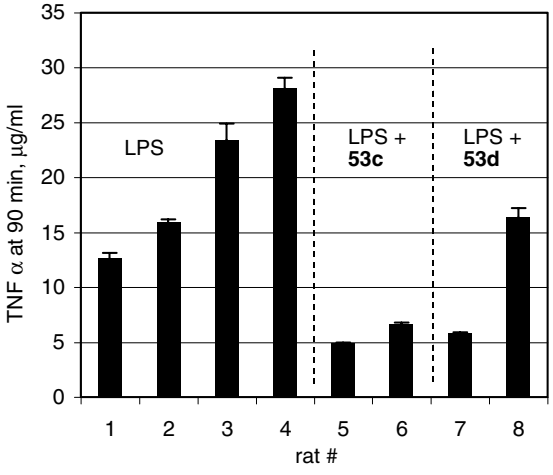


Fig. 10. Demonstration of in vivo efficacy (rat model) of the septic shock ameliorating effects of the tannin analogues **53c** and **53d**.

subjects. Monitoring the physiological symptoms of septic shock provided mixed results. Arterial blood pressure and plasma glucose levels were not significantly different between tannin treated and untreated rats. However, heart rates dropped to control levels upon administration of the tannin analogue **53c** (average beats/min for LPS treated, 483 ± 10; LPS + **53c**, 385 ± 33; saline control, 380 ± 22). These limited examples do not allow generalizations to be made, but, at the very least, they do provide encouragement for further study of the dimeric tannin analogues' structure/activity profile in anti-sepsis assays.

6. Conclusions and future prospects

The assembly of the naturally occurring dimeric ellagitannin coriariin A (**1**) and the preparation of the C(2)/C(4)-HHDP-containing glucopyranose system define the frontiers of our synthesis efforts in this area. Whereas fashioning the HHDP group appears to be a solved problem, both digalloyl ether and DHHDP formation are not. These key ellagitannin structural units can be prepared in simple systems, but exporting those advances to more realistic ellagitannin targets remains problematic. The great diversity of galloyl coupling motifs amongst the ellagitannins (e.g., valoneoyl, sanguisorboyl, macaranoyl, etc.) is a reminder that challenges for total synthesis still abound, and the development of new chemistry to address these challenges will surely be required.

The role that ellagitannins play in immunomodulation is beginning to be probed at the molecular level. Three important observations have emerged so far: (1) dimeric ellagitannins elicit secretion of TNFα from hPBMCs, (2) galloyl coupling to form an HHDP unit is not essential for this capability, and (3) the digalloyl ether linker unit is required for activity. Antagonists of lipid A's sepsis-inducing activity can be prepared based on the dimeric tannin platform and an untested mechanism-of-action model. These species constitute perhaps a new lead in the development of anti-sepsis chemotherapy agents, and, as such, help expand the potential utility of the ellagitannin family of secondary plant metabolites into a new sector of human health concerns.

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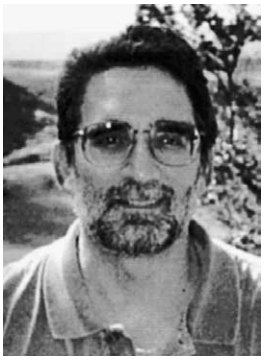

Ken Feldman graduated from Harvey Mudd College in 1978 with a Bachelor's of Science degree. In 1983 he completed his Ph.D. research at Stanford University under the direction of Dr. E.E. van Tamelen, after which he spent a one-year post-doctoral fellowship in the Central Research Department at Dupont. He began his independent academic career in 1984 as an Assistant Professor in the Chemistry Department at Pennsylvania State University, and he now holds the rank of Professor in that same Department. His major research interests lie in the area of organic synthesis, focused of late in the areas of ellagitannin chemistry and marine alkaloid chemistry.