Isolation and structure determination of new neolignans and cabagranin D, an unusual meroterpenoid from *Piper cabagranum*

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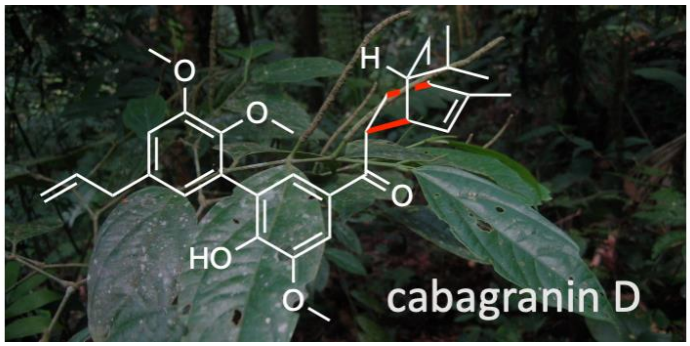


ABSTRACT: A novel meroterpenoid cabagranin D was isolated along with related neolignans cabagranins A-C from the leaves of *Piper cabagranum* (Costa Rica). Cabagranins A-C represent the first examples of 3,3'-neolignans isolated from the Piper genus of plant and the meroterpenoid cabagranin D, representing an unprecedented Diels-Alder conjugate of an unsubstituted phenylpropenone and α-phellandrene. Details of the full structural elucidation of these compounds and a discussion of the potential biosynthetic relationships are presented.

The Piper genus of plants (*Piperacae*) is known for the diversity of compounds that have been isolated from the over 2,600 accepted species that are distributed across the tropics.[1–6] Numerous studies have characterized the role of these compounds in various ecological interactions and have uncovered novel compounds with a wide diversity of biological activities, including antimicrobial and anti-herbivore activity.[7]

In a phytochemical survey of Piper species within the Radula clade, we identified *Piper cabagranum* as having unique chemistry based on GC-MS and ^{1}H NMR analysis of crude extracts. We observed that while general categories of natural products like lignans, sesquiterpenes, flavonoids, and kava lactones were consistently found among closely related species, ^{1}H NMR analysis of crude leaf extracts revealed that specific structural motifs varied widely.[8,9] This divergence in functional motifs likely stemmed from distinct evolutionary paths of these plant species, creating fertile ground for the discovery of new natural products. Through the variety of analytical analyses, we were able to pinpoint unique spectral characteristics within the crude methanolic extract of *Piper cabagranum* (Costa Rica), setting it apart from the other 70 species within our study, thus motivating our pursuit of the full phytochemical characterization of these compounds with the broader goal of understanding the role of these metabolites in mitigating ecological interactions. Isolation and characterization of leaf extracts from *Piper cabagranum* resulted in the discovery of an unprecedented meroterpene Diels-Alder conjugate cabagranin D, **5** (Figure 1). Furthermore, this work identifies a new series of related dehydrodieugenol type

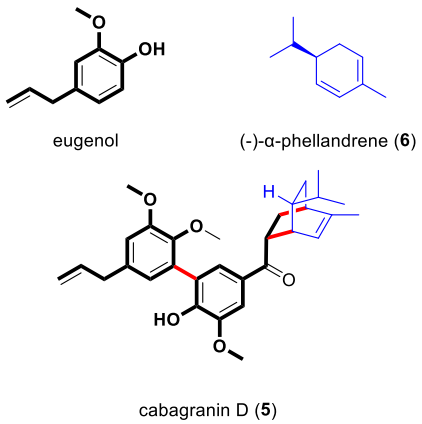


Figure 1. Structure of the novel meroterpenoid **5** isolated from *Piper cabagranum*

3,3'-neolignans (cabagranins A-C; **1**-**3**), which implicate the novel biosynthetic connections between cabagranin D (**5**) and the co-isolated neolignans (Figure 2). Herein, we report the isolation, structural, and stereochemical characterization of new meroterpenoid **5** and neolignans **1**-**3**, natural products from *Piper cabagranum* (Costa Rica).

Air dried and powdered leaf material of *P. cabagranum* from our field studies in Costa Rica were extracted with methanol and fractionated over a SepPak C18 column using varying proportions of acetone:water. Fractions eluted with 50% acetone:water, and subsequent purification via reversed-phase preparatory HPLC (C18) resulted in the isolation of cabagranin A (**1**) as a colorless oil, which was found to have the formula $C_{21}H_{24}O_5$ from HRESIMS m/z = 379.1551 $[M+Na]^+$, corresponding to an oxygenated dehydrodieugenol derivative. ^{1}H NMR analysis revealed the clear presence of a *bis*-phenylpropanoid, with two different propenyl units, one of which was substituted at C-7 as indicated by resonance δH 5.07 ppm (d, *J* = 5.6 Hz)/δC 76.0 ppm, which was coupled to the C-8 vinylic methine δH 6.05 ppm (ddd, *J* = 17.1, 10.3, 5.9 Hz)/δC 142.3 ppm via COSY and HMBC analysis. The three different methoxy singlets were assigned as aryl methyl ethers based upon HMBC correlations to quaternary oxygenated aromatic carbon resonances (Figure 2).

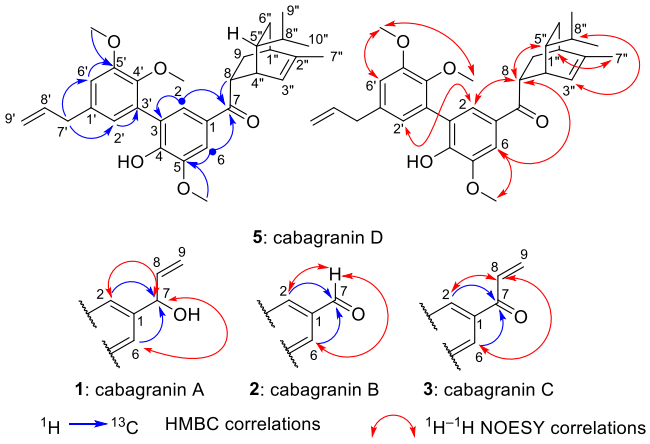


Figure 2. 2D NMR correlations establishing the proposed structures and relative configuration of cabgranins A-D

Proton resonances in the aromatic region indicated the presence of two pairs of *meta*-coupled protons (δH 6.96/6.74 ppm and δH 6.83/6.65 ppm, *J* ~ 2 Hz), each pair displaying HMBC correlations with two aromatic *O*-substituted carbons (δC 144–154 ppm) and one of the benzylic carbons (δC 76.0 ppm and δC 40.8 ppm, respectively). NOESY correlations between the most shielded protons in each ring supported the proximity of the two rings through direct linkage. We believe that the two rings non-coplanar orientation of the biphenyl groups anisotropically shields the protons H2/H2'. Lastly, NOESY correlations were used to assign location of three methoxy groups across the aromatic rings, which supported that the lone phenol was *para* to the modified propenyl moiety. All attempts to evaluate the enantiopurity of cabagranin A (**1**) and assign the absolute configuration of the alcohol using Mosher ester analysis were unsuccessful due to decomposition of the material under a variety of esterification conditions. ECD analysis demonstrated no Cotton effect suggesting that cabagranin A was isolated as a racemate. It was later found that the labile alcohol rearranged to the conjugated cinnamyl alcohol upon standing in solution over months at room temperature, or treatment with aqueous acid. This suggests that the secondary alcohol could be racemizing even if it is being produced as a non-racemic mixture in the organism through reversible elimination of water through a vinyl quinone methide intermediate. Overall, this analysis led to the assignment of novel neolignan cabagranin A (**1**) as the major component of the crude extract.

Further separation of the 70% acetone fraction resulted in four minor components that retained many of the structural features present in the related compounds, including methyl dehydrodieugenol (**4**, Scheme 1), which is presumed as the biosynthetic precursor to cabagranin A (**1**), and a known coumarin. Two new compounds were also isolated from this fraction, which were assigned as cabagranin B (**2**) and cabagranin C (**3**) bearing the identical *bis*-aryl phenol moiety of cabagranin A, but differing in their modified propenyl moieties with cabagranin B containing an aldehyde substituent (**2**: R = -CHO) and cabagranin C containing the 1-propenone substituent [**3**: R = C(=O)CH=CH_2] (Figure 2). It is important to note that neolignans containing the vinyl ketone substituent of **3** have only been isolated in a few cases and most reports suggest this product is the result of lignin decomposition.

Cabagranin D (**5**) was isolated as the predominant component of the hexane extract and found to have the formula $C_{31}H_{38}O_5$ from HRESIMS. 1D and 2D NMR spectral analysis indicated the presence of the 3,3'-biaryl structure analogous to cabagranins A-C in addition to an *iso*-propyl group (0.85 ppm), an allylic methyl (δH 1.76 ppm and δC 20.0 ppm), and a vinylic proton [δH: 5.49 ppm (dt *J* = 6.5 and 2.0 Hz) δC: 121.9 ppm]. ^{1}H–^{1}H COSY correlations were supportive of a [2.2.2] bicyclic structure, which were further supported by key HMBC correlations. HMBC correlation between the C-2 and C-6 aryl methines and the C-8 methine with the carbonyl carbon at δC 202.4 ppm revealed that this 12-carbon unit was connected to the aromatic ring through the C-7 ketone group (Figure 2). The relative configuration of the bicyclic stereogenic centers at C-8 and C-5" were assigned from NOESY correlations between H-8 to H-5" and H-8" to H-3". Further 2-D NMR correlations were consistent for the structural assignment of cabagranin D, which is postulated to be the *endo* product of Diels-Alder coupling between the enone of cabagranin C (**3**) and the monoterpene α-phellandrene (**6**, Figure 1). To the best of our knowledge, this new molecule represents a novel late-stage merger between a terpene and a neolignan, presumably through a Diels-Alder reaction.

Given that both enantiomers of α-phellandrene have been isolated from natural sources, we could not imply the absolute configuration of cabagranin D, thereby motivating our evaluation of the absolute configuration by experimental and computation ECD analysis. The ECD spectrum of cabagranin D (**5**) showed strong Cotton effects at 250, 290 and 330 nm. Simulation of the ECD spectra using time dependent density functional theory (TDDFT) calculations (M06/6-31G+*) of energy-minimized structures of both enantiomers of cabagranin D in an implicit solvent model (PCM) for methanol strongly aligned with the UV absorbances and sign

corresponding to an *endo* cycloaddition of R-(-)-α-phelladrene (**6**) with cabagranin C from the face opposite of the bulky *iso*-propyl substituent confirming the assignment of the absolute configuration of cabagranin D.

Table 1. ^{1}H and ^{13}C NMR data for cabagranin D (5).

	cabagranin D (5 in CD_3OD)	
position	**^{1}H (*J* in Hz)**	**^{13}C**
1		128.8
2	7.44 (1H, d, 2.0)	126.5
3		133.1
4		150.2
5		149.1
5-OMe	3.95 (3H, s)	56.6
6	7.48 (1H, d, 2.0)	110.8
7		202.4
8	3.50 (1H, ddd, 9.4, 5.8, 1.9)	48.4
9	1.77-1.70 (2H, m)	29.5
1'		137.2
2'	6.67 (1H, d, 2.0)	124.2
3'		126.5
4'		146.4
4'-OMe	3.60 (3H, s)	61.0
5'		113.6
5'-OMe	3.88 (3H, s)	56.3
6'	6.87 (1H, d, 2.0)	154.0
7'	3.37 (2H, br d, 6.8)	41.0
8'	5.99 (1H, ddt, 16.9, 10.0, 6.7)	138.9
9'-*cis*	5.05 (1H, dq, 10.0, 2.0)	116.0
9'-trans	5.11 (1H, dq, 17.0, 2.0)	
1"	2.40 (1H, m)	37.5
2"		144.6
3"	5.49 (1H, dt, 6.2, 1.7)	121.9
4"	2.93 dt (1H, 6.5, 2.0)	38.7
5"	1.48 (1H, m)	48.5
6"-α	1.80 (1H, m)	32.7
6"-β	0.97 (1H, m)	
7"	1.76 (3H, d, 1.7)	20.0
8"	1.08 (1H, m)	34.5
9"	0.88 (3H, d, 6.5)	21.7
10"	0.82 (3H, d, 6.6)	20.9

The isolation of a series of neolignans **1**-**4** lends support to the proposed biosynthetic pathway shown in Scheme 1. This hypothesis suggests that eugenol undergoes oxidative dimerization to generate dehydrodieugenol, which is subsequently monomethylated to yield compound **4**. The major constituent of the crude extract is formed through the selective oxidation of the propenyl substituent attached to aryl ring containing the free phenol. A variety of neolignans have been isolated from other Piper species, however, this represents the first example containing an oxidized propenyl side chain.[10] While the conversion of the alcohol to ketone **3** is anticipated to be facile, the aryl vinyl ketone's high electrophilic reactivity, its exceptionally rare occurrence in natural products, and the observed transformation of the alcohol in cabagranin A to cabagranin C during storage, collectively suggest that the free enone might not always be present in detectable concentrations within the plant.[11–13] This could potentially result from the instability of the vinyl ketone and its presumed toxicity as a covalent modifier of biomolecules.[11–13]

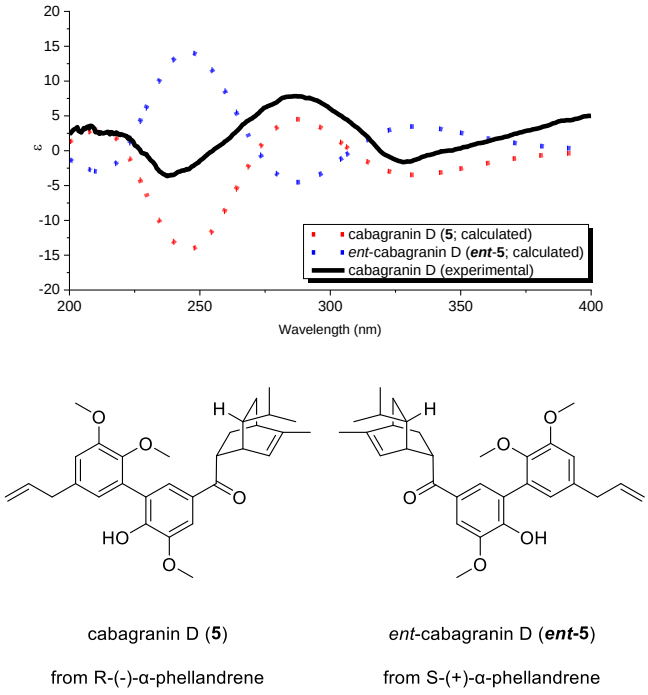


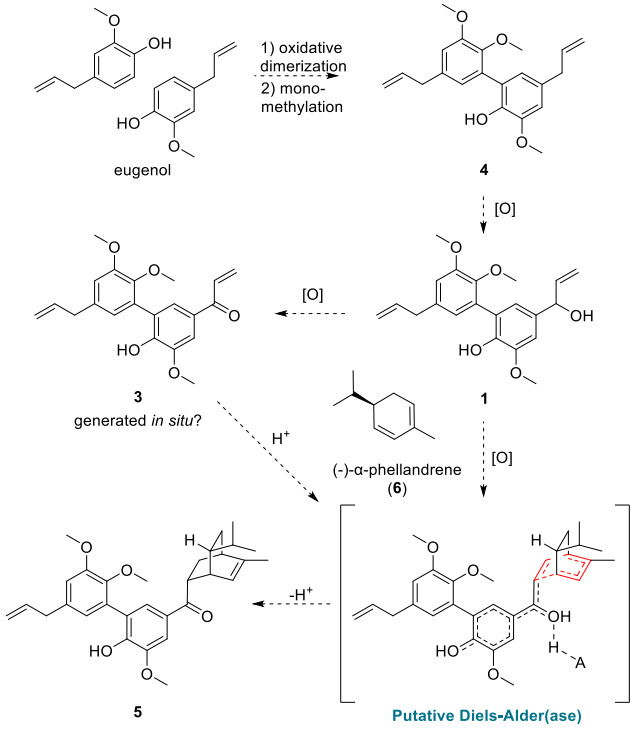
Figure 3. Comparison of the calculated ECD spectra of both enantiomers of cabagranin D using time dependent density functional theory (TDDFT) calculations and experimental ECD spectra

However, the discovery of meroterpenoid cabagranin D (**5**) implies that the reactive ketone is effectively captured in a Diels-Alder reaction with α-phellandrene (**6**). While there are a few examples of phellandrene or terpene conjugates of beta-substituted aryl enones, this finding establishes the first instance of an unsubstituted phenylpropenone-terpene Diels-Alder conjugate, implying the presence of compound **3** *in-vivo*.[14–18] Alternatively, an *in-situ* generation of the reactive enone **3** is hypothesized, accompanied by its immediate trapping by α-phellandrene, possibly facilitated by a single enzymatic step. In this scenario, the Diels-Alder reaction could emerge from the activity of an oxidase enzyme acting on the alcohol within compound **1**. This oxidation of cabagranin A would lead to the formation of a vinyl para-quinone methide intermediate, representing the protonated form of the vinyl ketone, which in the presence of α-phelladrene, would produce cabagranin D. The roles of various classes of enzymes in catalyzing Diels-Alder reactions has been established.[19,20] Recent research highlights the role of redox-active enzymes that have likely diverged from their ancestral functions to catalyze

Diels-Alder reactions in the biosynthesis of prenylated phenol and indole natural products.[21,22] Other investigations have shown that oxygenated aromatic groups can act as redox tags in electrocatalytic Diels-Alder reactions and that silver nanoparticles can catalyze related Diels-Alder reactions involving phenolic chalcones and terpenoid dienes.[23,24] Given the interconnectedness of the compounds isolated in this study and their apparent biosynthetic relationships, the findings support the notion that a lignan oxidase might play a role in facilitating the Diels-Alder reaction between the enone (**3**) and α-phellandrene (**6**). While the concurrent presence of the compounds from this study and evidence of redox-active Diels-Alderases support this hypothesis, we cannot exclude the possibility of traditional Lewis-acid catalysis or single-electron processes catalyzing the proposed Diels-Alder reaction. Ongoing experimental and computational investigations are underway to evaluate our biosynthetic hypothesis surrounding the proposed Diels-Alder step leading formation of cabagranin D (**5**).

Piper cabagranum is a chemically distinct species when compared across various members of the genus because of the presence of oxidized 3,3'-neolignans and the unique neolignan meroterpenoid that represents the first example of a Diels-Alder conjugate of an arylvinyl ketone dienophile with a terpene diene. The unique structure of **5**, the co-occurrence of the major component **1**, the scarcity and reactivity of vinyl ketones, suggests that the biosynthetic Diels-Alder reaction to produce **5** is occurring by an *in-situ* generation of the reactive dienophile **3**. This discovery implicates a new class of redox active Diels-Alderase and prompts further investigations.

Scheme 1.



ASSOCIATED CONTENT

Supporting Information

Copies of 1D and 2D NMR spectra for cabagranins A-D (**1**-**3, 5**), methyl dehydrodieugenol (**4**), known coumarin, and computational details for the ECD calculations are provided in the supporting information.

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