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

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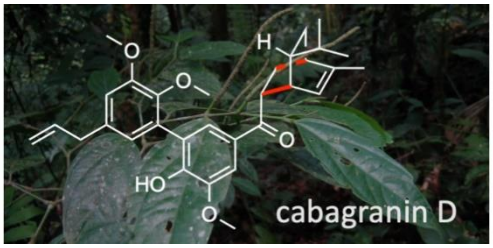
*Supporting Information Placeholder*




**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 plant genus *Piper*, and the meroterpenoid cabagranin D displays 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 the source of a diversity of compounds that have been isolated from over 2,600 accepted species 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.[8] We observed that general categories of natural products like lignans, sesquiterpenes, and flavonoids were shared among closely related species, however $^1$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. The unique spectral features encountered in the crude methanolic extract of *Piper cabagranum* (Costa Rica) set it apart from the other 70 species within our study and motivated the phytochemical characterization of this species with the goal of understanding the role of specialized metabolites in mediating ecological interactions. Our work led to the discovery of an unprecedented meroterpene Diels-Alder conjugate cabagranin D, **5** (Figure 1). Furthermore, this work identifies a new series of dehydrodieugenol derived 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).

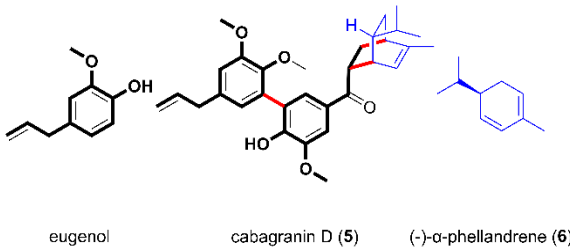



**Figure 1.** Structure of the novel meroterpenoid **5** isolated from *Piper cabagranum.*

Air dried and powdered leaf material of *P. cabagranum* from our field studies at La Selva Biological Station (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 subsequently purified via reverse-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 differing propenyl units. One of these units was hydroxylated at C-7 as indicated by the resonance $\delta_H$ 5.07 (d, $J$ = 5.6 Hz)/$\delta_C$ 76.0, which was coupled to the C-8 vinylic methine $\delta_H$ 6.05 (ddd, $J$ =

17.1, 10.3, 5.9 Hz)/$\delta_C$ 142.3 based on COSY and HMBC analyses. HMBC correlations to quaternary oxygenated aromatic carbons led to the assignment of the three different methoxy singlets as aryl methyl ethers (Figure 2).

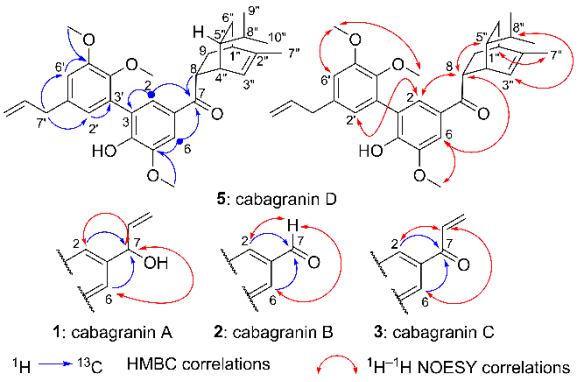



**Figure 2.** 2D NMR correlations establishing the proposed structures and relative configuration of cabgranins A-D.

Proton resonances in the aromatic region indicated the presence of two pairs of *meta*-coupled protons ($\delta_H$ 6.96/6.74 and $\delta_H$ 6.83/6.65, $J$ ~ 2 Hz), each pair displaying HMBC correlations with two aromatic *O*-substituted carbons ($\delta_C$ 144–154) and one of the benzylic carbons ($\delta_C$ 76.0 and 40.8, respectively). NOESY correlations between the most shielded protons in each ring supported the proximity of the two rings through direct linkage. 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.

Attempts to evaluate the enantiopurity of cabagranin A (**1**) and assign the absolute configuration of the alcohol were unsuccessful due to decomposition of the material under a variety of derivatization conditions. ECD analysis demonstrated no Cotton effects suggesting that **1** was isolated as a racemate. We found that the labile alcohol rearranged to a 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 naturally produced as a non-racemic mixture.

Separation of the 70% acetone fraction resulted in four minor components that retained most of the structural features present in **1**, including **4** which is presumed as the biosynthetic precursor to **1**, and a coumarin (Scheme 1, see SI). Two new compounds were isolated from this fraction, bearing the identical *bis*-aryl phenol moiety of **1**, but differing in their modified propenyl moieties. These compounds were assigned as cabagranin B (**2**), containing an aldehyde substituent, and cabagranin C (**3**), which contains a 1-propenone substituent (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 pyrolysis.

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 m/z = 513.2653 $[M+Na]^+$. NMR spectral analysis indicated the presence of the 3,3'-biaryl structure analogous to **1**-**4** in addition to an *iso*-propyl group ($\delta_H$ 0.85), an allylic methyl ($\delta_H$ 1.76 and $\delta_C$ 20.0), and a vinylic proton [$\delta_H$ 5.49 (dt, $J$ = 6.5 and 2.0 Hz), $\delta_C$ 121.9]. $^1$H–$^1$H COSY correlations were consistent with a [2.2.2] bicyclic structure, which was supported by key HMBC correlations between the H-2 and H-6 aryl methines and the H-8 methine with the carbonyl carbon at $\delta_C$ 202. The relative configuration of 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 **5**, which is postulated to be the *endo* product of a Diels-Alder coupling between the enone of **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.

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

| Position | cabagranin D (5, in $CD_3OD$) | |
|---|---|---|
| | $^1$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 |

The ECD spectrum of **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 *iso*-propyl substituent, thus confirming the assignment of the absolute configuration of **5**.

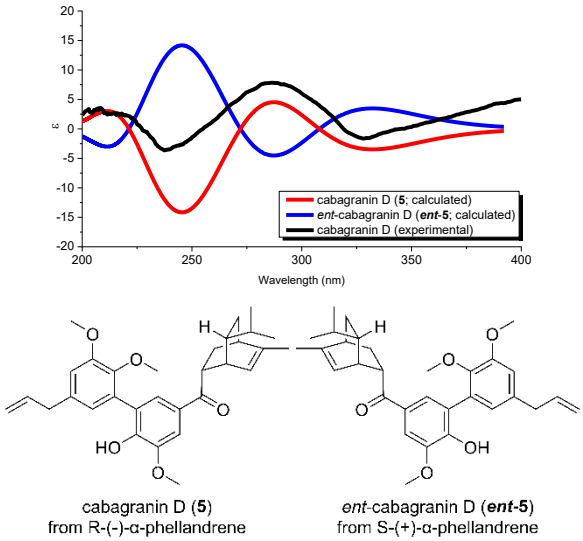


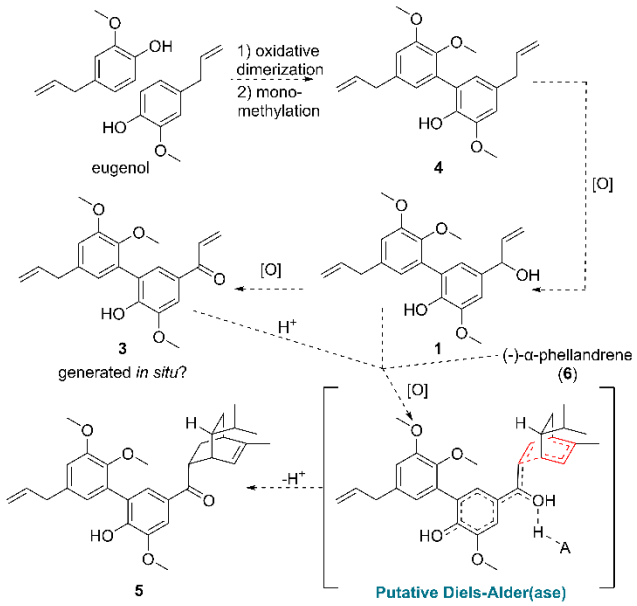

**Figure 3.** Comparison of the calculated ECD spectra of both enantiomers of cabagranin D using time dependent density functional theory (TDDFT) calculations and experimental ECD spectra.

The co-isolation of the series of neolignans **1**-**4** lends support to the proposed biosynthetic pathway shown in Scheme 1. This hypothesis suggests that eugenol undergoes oxidative dimerization followed by monomethylation to yield compound **4**. The major constituent of the crude extract is formed through the selective oxidation of the propenyl substituent of the phenolic ring. A variety of neolignans have been isolated from other *Piper* species, however, this represents the first example containing a hydroxylated propenyl side chain.[10] While the conversion of the alcohol to ketone **3** is anticipated to be facile, the free enone was not always present in detectable concentrations in the crude extracts of the leaves. The high electrophilic reactivity of **3**, its rare occurrence,[11–13]and presumed toxicity as a covalent modifier of biomolecules suggest that this compound could be an artifact of isolation and not present in high concentrations *in-vivo*.[11–13]

The discovery of the meroterpenoid **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 β-substituted aryl enones, the isolation of **5** establishes the first example of a Diels-Alder product between an unsubstituted phenylpropenone and a terpene.[14–18] Given the instability of **3**, we hypothesize that the ketone precursor could be formed *in-situ* and simultaneously trapped by α-phellandrene in a single enzymatic step. In this scenario, the Diels-Alder product could emerge from the activity of an oxidase enzyme acting on the alcohol within compound **1**. This oxidation of **1** would lead to the formation of a vinyl *p*-quinone methide intermediate, representing the protonated enone, which would produce **5** (Scheme 1) from reaction with α-phellandrene. Recent research highlights the role of redox-active enzymes that have likely diverged from their ancestral functions to act as Diels-Alderases in the biosynthesis of prenylated phenol and indole natural products.[21,22] Other investigations have shown that phenols and their ethers 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] While these reports support our hypothesis, we cannot exclude the role of Lewis-acid or single-electron processes in catalyzing the proposed Diels-Alder reaction. Ongoing experimental and computational investigations are evaluating our biosynthetic hypothesis surrounding the formation of **5**.

The compounds isolated in this study establish *Piper cabagranum* as a chemically distinct species within its genus, primarily due to the presence of oxidized 3,3'-neolignans and a distinctive neolignan meroterpenoid, cabagranin D, marking the first occurrence of a Diels-Alder between a vinyl ketone dienophile and a terpene diene, and inspiring future studies on the biosynthetic origins of this unique compound.

**Scheme 1.**




## ASSOCIATED CONTENT

### Supporting Information

Details of the isolation and purification of all compounds, 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 (PDF)

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